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ANNUAL PROGRESS REPORT FY 1970

RCS-MEDDH-288(RI)

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FORT DETRICK, MARYLAND

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U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FORT DETRICK, FREDERICK, MARYLAND 21701

ANNUAL PROGRESS REPORT

FISCAL YEAR 1970

RCS-MEDDH-288(R1)

Project 1B662706A096

1 July 1970

SUMMARY

A report of progress on the research program of the U. S. Army Medical Research Institute of Infectious Diseases on Medical Defense Aspects of Biological Agents (U) for Fiscal Year 1970 is presented.

FOREWORD

This FY 1970 Annual Progress Report is a general review of research activities of the U. S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, conducted under Project 1B662706A096, Medical Defense Aspects of Biological Agents (U). The project is divided into three tasks:

1B662706A096 01 - Pathogenesis of Infection of Military Importance.
1B662706A096 02 - Prevention and Treatment of Biological Warfare Casualties.
1B662706A096 03 - Laboratory Identification of Biological Agents.

Twelve contracts are currently in effect with educational institutions or industrial firms. Reports are available through DDC.

Tasks are subdivided into work units, each identified by a three digit suffix. Numbers have been assigned in accordance with the following scheme:

General	001-099
Bacterial Diseases	100-299
Rickettsial Diseases	300-399
Viral Diseases	400-699
Mycotic Diseases	700-799
Intoxications	800-899
Contracts	900-999

Three appendices are included covering the Guest Lecture Series, Professional Staff Meetings, Formal Presentations and Briefings and a list of publications of the Institute for the Fiscal Year. An index by authors has been added.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resourses, National Academy of Sciences-National Research Council.

1 July 1970

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ANNUAL PROGRESS REPORT -- FY 1970

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Task No. 1B662706A096 01:	**Pathogenesis of Infection of Military Importance**
Work Unit No. 096 01 001:	**Metabolic Studies in Experimental Disease**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Physical Sciences and Medical
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Kenneth A. Woeber, Lt Colonel, MC (I, III) Peter J. Bartelloni, Lt Colonel, MC (I, III) William R. Beisel, M.D. (I, III) Gordon L. Bilbrey, Major, MC (II) William A. Harrison, Captain, VC (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

2

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0800	70 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	001
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Metabolic studies in experimental disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	3	20
FISCAL YEAR CURRENT 71	3	20

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: Beisel, W. R. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Sandfly fever; (U) Metabolism; (U) Work performance; (U) Nitrogen balance; (U) Enzymes; (U) Renal function; (U) Acid base balance

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study early changes in metabolism induced by experimental infection.

24 (U) A variety of techniques are employed to study metabolic changes associated with infection.

25 (U) 69 07 - 70 06 - Metabolic and biochemical responses and work performance during sandfly fever infection were studied in volunteers. Nitrogen balance did not appear to correlate with decrements in work and muscular endurance. Muscle and neutrophil enzymes were measured concurrently. No clear-cut pattern of response was observed for the former. However, there were extensive alterations in neutrophil enzymes within inidividual cells indicative of inhibited pentose glycolytic pathway function.

In renal function studies, it was found that acute pneumococcal bacteremia in rhesus monkeys increased glomerular filtration rate, effective renal plasma flow, urinary volume, and clearance of free water.

A one-time study of acid-base balance measurement was done. Respiratory alkalosis was the usual early response of the human host to sandfly fever.

Publication: Fed. Proc. 29:494, 1970 (abstract).

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 001: Metabolic Studies in Experimental Disease

Description:

To study early changes in metabolism induced by experimental disease.

Progress, Part I:

During the present fiscal year, studies to define metabolic and biochemical changes during infectious illnesses in man were continued during a series of 3 studies dealing with experimentally induced sandfly fever (Project Numbers FY 70-1, FY 70-2, and FY 70-3). The first of these studies (Project No. FY 70-1) was performed in conjunction with Dr. Earl Alluisi and his research group at the University of Louisville (Contract No. DA 49-193-MD-2567) and the U. S. Army Medical Research Laboratory, Fort Knox, Kentucky. Work performance and muscular function were measured during the infection; an attempt was made to interrelate observed changes with alterations in clinical, biochemical and metabolic responses. Accordingly, the study was devised in such a manner that the dietary intake could be monitored with accuracy. To facilitate this aspect of the study, Captain Carol M. Johnson, AMSC, Research Dietitian of the Department of Metabolism, Walter Reed Army Institute of Research, planned a daily menu for the entire study period and supervised the meal-by-meal distribution of weighed servings of food to the volunteers. The exact dietary intake of each volunteer was recorded for later calculations. In this study, nitrogen balance became negative in conjunction with the onset of symptomatic illness; cumulative losses of nitrogen progressed in a stepwise fashion until 8 days postinoculation. The average loss of body nitrogen in volunteers with sandfly fever amounted to 66 gm. This loss could be accounted for by reduction in dietary intake in conjunction with an unchanged or slightly decreased loss of urinary nitrogen. There was no increase in excretion of urinary amino acid nitrogen, uric acid, or urea. As in earlier studies, the excretion of urinary Na and Cl showed a reduction following the onset of symptoms. A reduction in the excretion of K, Ca, and Mg was also noted, while phosphorus values tended to fluctuate near baseline. When analyzed on an individual basis, the changes in nitrogen, mineral, or electrolyte balance did not appear to correlate with the measured decrement in work performance observed within individual subjects. Muscle function changes revealed a minimal and variable decrement of muscle strength and a greater

decrement in muscular endurance during illness. None of the muscle function changes, however, could be correlated in individual subjects with the magnitude of fever and clinical illness or with the degree of change in nitrogen metabolism.

In this and another study (Project No. FY 70-3), serum concentrations of the muscle enzymes aldolase and creatinine phosphokinase were measured. Enzyme activity in serum generally tended to remain within normal limits during sandfly fever, although increases were noted in occasional subjects. Changes in both enzymes did not always occur in serum from the same individual and the overall pattern of change did not appear to correlate in individual volunteers with the degree and severity of illness, other biochemical findings, work performance data, or with changes in muscle function.

To investigate serial changes in white blood cell enzymes, a cooperative study was conducted with Dr. Joseph A. Bellanti at Georgetown University. During the course of the FY 70-2 sandfly fever study, white blood cells were harvested from heparinized blood, and a quantitative measurement of the dye nitroblue tetrazolium (NBT), and enzymes glucose-6-phosphate dehydrogenase (G-6-PD), and 6-phosphogluconic dehydrogenase (6-PGD) was performed. In each of 6 volunteers with sandfly fever, a decrease in quantitative NBT reduction was observed. This occurred between the 4th to the 7th day and levels as low as 20% of baseline values were observed. In contrast, similar changes were not observed in the controls. The values all returned to normal by the 19th day. A reduction in G-6-PD activity was observed in all infected volunteers. This was usually detected by the 7th day; activities as low as 5 to 6% of baseline values were observed in some of the men in contrast to the controls who failed to show similar changes. G-6-PD activity did not return to baseline values in 5 of 6 men for as long as the 19th day, the last day of testing. The activity of 6-PGD did not show changes in either controls or infected subjects. All observed changes involved calculations based on values for individual cells. The overall extent of change was magnified still further by the occurrence of typical neutropenia during sandfly fever.

In study FY 70-2, an attempt was made to estimate changes in vitamin metabolism. During these sandfly fever studies, analyses of whole blood and plasma folate were performed in a cooperative study by Drs. C. E. Butterworth, Jr. and T. W. Sheehy of the University of Alabama. The data on whole blood and plasma concentration of folate provided no evidence of any infection-related alterations. In a subsequent study (Project No. FY 70-3), additional volunteers were placed on a constant intake of vitamins and trace minerals as well as other dietary constituents for an extended period of time prior to the initiation of experimental sandfly fever. In conjunction with investigators at the U. S. Army Medical Research Nutrition Laboratory (COL J. E. Canham, MC, COL R. H. Herman, MC, Dr. H. Sauberlich, and Mrs. Yaye Herman), blood and urinary vitamin values were measured serially. No significant changes were noted in measurements of vitamins in blood. Vitamin losses via the urine are currently being analyzed.

Summary, Part I:

Studies of metabolic and biochemical responses of volunteers to sandfly fever were continued. Nitrogen balance changes in individual volunteers did not appear to correlate directly with a decrement in work performance or with a decrease in muscular endurance. Alterations in muscle enzymes were observed during sandfly fever, but did not follow a clear-cut pattern. There were measurable *in vitro* changes within the neutrophil during the course of this experimental viral infection. These consisted of changes in NBT dye reduction and G-6-PD function, variations indicative of impaired hexose monophosphate (HMP) activity. These changes appeared to be valid since each of the measurements were standardized to a fixed number of cells or to a fixed protein concentration. No changes in vitamin concentrations in blood or plasma have been observed during acute sandfly fever.

Progress, Part II:

The influence of acute infection on renal function was studied in 32 rhesus monkeys. All monkeys were anesthetized and studied over an 11-hr period under conditions of progressive volume expansion by hypotonic saline solution. Significant and immediate increases in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) occurred when live *Diplococcus pneumoniae* were injected intravenously; using heat-killed organisms, GFR increased significantly but not ERPF. By contrast, a downward trend of both parameters was noted in sham-inoculated saline controls, during artificial hyperthermia, and in monkeys studied 16 hr after inoculation with live organisms.

Other significant parameters altered were urinary volume and clearance of free water; both were significantly increased in the animals receiving live pneumococci. The monkeys with induced hyperthermia had a tendency to conserve both volume and free water.

The data suggest that significant decreases in renal resistance occur with live organisms, resulting in increased GFR and ERPF. The mechanism of these changes is unknown, but the release of leukocytic endogenous pyrogen is a possibility. Since live pneumococci caused free water formation and urine volume to increase, whereas artificial hyperthermia had the opposite effect, (presumably as a result of antidiuretic hormone release) it seems possible that an even greater increase in free water and volume excretion would occur in infected animals if hyperthermia were prevented during the infection.

Summary, Part II:

Preliminary studies in rhesus monkeys indicate that acute pneumococcal bacteremia increases glomerular filtration rate, effective renal plasma flow, urinary volume, and free water clearance. The mechanisms underlying these alterations are currently under study.

Progress, Part III:

The sequential changes in acid-base balance during infection in man have not been established. Available data indicate that respiratory alkalosis is the usual early host response to acute infection; this is accompanied by increases in blood pyruvate and lactate, as well as by decreases in urinary and serum inorganic phosphate.

Measurements of blood pyruvate, lactate, PO_2, PCO_2, pH and inorganic phosphate were obtained in 8 men during an induced sandfly fever infection (Project No. FY 70-2). Arterialized capillary blood from the finger tip was used for the measurement of PO_2, PCO_2 and pH.

The results suggest that respiratory alkalosis, as judged from a decrease in PCO_2 and increase in pH, occurs at the onset of fever. Since pyruvate and lactate levels fluctuated widely, it was not possible to draw any conclusions. Plasma inorganic phosphate showed no significant variation during the illness. No further work is contemplated.

Summary, Part III:

Preliminary data obtained during sandfly fever infection confirm previous work suggesting that respiratory alkalosis is the usual early host response to symptomatic infectious illness.

Presentation:

Bilbrey, G. L. Renal function in acute pneumococcal infections. Presented at National Meeting, American Federation for Clinical Research, Atlantic City, N. J., 2-3 May 1970.

Publication:

Bilbrey, G. L., R. T. Vollmer, and W. R. Beisel. 1970. Renal function in acute pneumococcal infections. Fed. Proc. 29:494 (abstract).

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 002:	Role of Hormones in Infectious Disease
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Physical Sciences & Medical
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Kenneth A. Woeber, Lt. Colonel, MC (I, II) John H. Boucher, Major, VC (III) John L. Winnacker, Major, MC (IV, V) Neil J. Grey, Major, MC (VI)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 0L0801	2. DATE OF SUMMARY* 70 07 01		REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	002
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Role of Hormones in Infectious Disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	4	30
FISCAL YEAR CURRENT 71	4	30

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Boucher, J. H. DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Thyroid hormones; (U) Kinetics; (U) Pneumococcus; (U) Catecholamines; (U) Insulin; (U) Encephalitis; (U) Sandfly fever; (U) Growth hormone; (U) Radioisotope

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Study the role of hormones in host response to infection.

24. (U) Isotope tracer techniques are employed to assess thyroid hormone economy and growth hormone and insulin levels in infection. Cardiac catheterization is employed to study myocardial dynamics during stress. A variety of chemical procedures are employed to study metabolic fuel interrelationships during infection.

25. (U) In the rhesus monkey, acute pneumococcal infection is accompanied by increased turnover of thyroxine and triiodothyronine. The binding of thyroxine in serum does not appear to change during the acute febrile phase of pneumococcal infection or sandfly fever. Preliminary data indicate that a significant pressure gradient develops across the aortic valve in thyrotoxic dogs stressed by immobilization. Plasma growth hormone increases in febrile illness. This increase, which does not appear to occur in non-infectious hyperthermia, cannot be suppressed by glucose but can be diminished by alpha adrenergic blockade with phenoxybenzamine. The role of endogenous pyrogen in evoking the growth hormone increase is under investigation. Studies designed to assess metabolic fuel interrelationships and insulin levels during infection are in progress.

Publication: American Thyroid Assoc. Program, p. 49, 1969.

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 002: Role of Hormones in Infectious Disease

Description:

Study the role of hormones in host response to infection.

Progress, Part I:

The effects of acute infection on thyroid hormone economy are uncertain. Although acute infection has been shown to influence thyroid function and the peripheral metabolism of L-thyroxine (T4) and L-triiodothyronine (T3) in several animal species, the results of such studies have tended to be conflicting, appearing to vary with the nature of the species examined. Accordingly, the present work was undertaken in order to assess in a primate host various aspects of thyroid hormone economy during progression from the healthy state through acute bacterial infection into convalescence. The rhesus monkey, Macaca mulatta, was employed as the host, and a virulent strain of Diplococcus pneumoniae, as the infecting agent.

The monkeys were secured in chairs and fed General Biochemicals Monkey diet which had an iodine content of 0.14 to 0.17 µg/gm. After a 3-week period of adaptation had elapsed, each monkey was given an intravenous (IV) injection of 25 µc of carrier-free inorganic ^{125}I to label the intra-thyroidal iodine pool, thereby permitting synthesis of endogenously labeled thyroid hormones. Serum protein-bound ^{125}I ($PB^{125}I$) was measured daily thereafter. Ten days later, when the values for serum $PB^{125}I$ had attained a virtual plateau, each monkey was given an IV injection of 10 µc of $T4\text{-}^{131}I$ to label the peripheral hormonal pool. Thereafter, serum protein bound ^{131}I ($T4\text{-}^{131}I$) was also measured daily. After a 5-day control period had elapsed, the monkeys were inoculated IV either with 3×10^8 D. pneumoniae organisms or with normal saline; this was followed immediately by a second IV injection of 10 µc of $T4\text{-}^{131}I$. Measurements of serum $PB^{125}I$ and $T4\text{-}^{131}I$ were obtained at 8-hourly intervals for the first 32 hr and daily thereafter. Five days later, the surviving monkeys were given a third injection of 10 µc of $T4\text{-}^{131}I$ and measurements were continued daily for a further 5 days.

The acute febrile phase of the illness had begun by 8 hr after inoculation and lasted from 48-72 hr. During this acute febrile phase, the calculated rate of clearance of $T4\text{-}^{131}I$ from the peripheral pool was significantly increased. Serum $PB^{125}I$ initially tended to decline, owing to

the increased rate of T4 clearance. Thereafter, serum $PB^{125}I$ increased to values in excess of control values, and this occurred in the face of accelerated T4 clearance, indicating that the rate of hormonal secretion had increased. During the convalescent phase, the rate of clearance of T4-^{131}I returned toward control values, but the increase in serum $PB^{125}I$ persisted. No alterations were observed in the sham-inoculated monkeys.

The binding of T4 in the sera of these monkeys was also studied. The sera were enriched with the equivalent of 64 µg of ^{131}I-labeled T4/100 ml and subjected to reverse flow filter paper electrophoresis in glycine-acetate buffer at pH 8.6. No changes in the distribution of T4 among its binding proteins were noted during the acute febrile phase, but during the convalescent phase, a greater than normal proportion appeared to be associated with the T4-binding globulin and a smaller than normal proportion with the T4-binding prealbumin.

Additional studies were undertaken to assess the influence of acute pneumococcal infection on peripheral T3 metabolism. Here, the monkeys were maintained on Purina Monkey Chow which had an iodine content of 1.6 µg/gm. Immediately after inoculation with D. pneumoniae or normal saline each monkey was given an IV injection of 5 µc of T3-^{125}I and 10 µc of T4-^{131}I. Measurements of serum protein-bound ^{125}I and ^{131}I were obtained over the next 5 days. As was the case with T4, the rate of T3 clearance also increased during the acute febrile phase.

These data indicate that the earliest detectable alteration following the initiation of infection is accelerated clearance of T4 and T3 from their respective peripheral pools. This alteration which could not be ascribed to a decrease in hormonal binding in serum was often evident by 8 hr and was accompanied later by a detectable increase in the rate of hormonal secretion.

Currently, the metabolism of T4 by tissues in vitro is being studied in an attempt to elucidate the mechanism of the alterations in peripheral hormonal metabolism observed in vivo.

Summary, Part I:

In the rhesus monkey, acute pneumococcal infection is accompanied by increased peripheral metabolism of both thyroxine and triiodothyronine which in turn is followed by an increase in the rate of hormonal secretion.

Progress, Part II:

The binding of L-thyroxine (T4) was studied in sera obtained from the volunteers who participated in sandfly fever Project No. FY 70-1. The binding capacity of the major T4-binding protein, T4-binding inter-α globulin

(TBG), was measured by subjecting sera enriched with 163 to 204 µg of T4-^{131}I/100 ml to reverse flow filter paper electrophoresis in glycine-acetate buffer at pH 8.6. In addition, the proportion of free T4 was assessed by the T3 resin uptake test (Triosorb-125, Abbott).

No alterations in the binding capacity of TBG or in the T3 resin uptake test were noted in sera collected during the acute febrile phase of sandfly fever. However, 3 of the volunteers displayed increased binding of T4 by TBG during the convalescent phase of illness.

Summary, Part II:

The physical state of thyroxine in serum does not change during the febrile phase of sandfly fever, but the convalescent phase may sometimes be accompanied by an increase in the binding capacity of thyroxine-binding globulin.

Progress, Part III:

The once generally regarded concept that the circulatory system is secondarily affected in thyrotoxicosis is no longer true today. The cardiovascular system has been shown to be in fact one of the primary effectors of the thyroid hormones. There are two presently accepted concepts which substantiate this. First, Brewster, et al.[1] suggested that the cardiovascular changes in thyrotoxicosis were the result of the thyroid hormones sensitizing the organism to its own endogenous catecholamines. Later, Buccino, et al.[2] showed that the thyroid hormone acts directly upon the myocardium to increase its contractility, independent of enhanced catecholamine sensitivity.

Previous studies[3] have shown that when a conscious thyrotoxic dog is stressed by immobilization the initial hypertension is followed by a progressive myocardial depression leading eventually to circulatory collapse and death. Accordingly, studies designed to explore the pathogenesis of the changes in myocardial dynamics in the thyrotoxic animal have been undertaken.

Three thyrotoxic and 2 control dogs have been used to measure serially left ventricular and aortic pressures for 6 hr following immobilization on a specially designed restraint board. A catheter was passed retrograde from the femoral artery into the left ventricle and pressures were recorded hourly during a catheter pull-back procedure into the aorta.

Thyrotoxicosis was induced acutely in the dogs by daily intramuscular administration of 1 mg/kg L-thyroxine (T4) and 0.75 mg/kg triiodothyronine (T3). After 7 days heart rates had doubled and pulse pressure had widened.

The thyrotoxic dogs were extremely hypertensive when stressed by immobilization. Without exception, the left ventricular peak systolic pressure was >250 mm Hg initially and progressively decreased to 200 mm Hg by the sixth hour. A pressure gradient of 60-90 mm Hg across the aortic valves

persisted throughout the 6-hr period of immobilization stress. Control dogs were also slightly hypertensive during the stressful period. However, left ventricular peak systolic pressure was always $\leq$ 190 mm Hg and the pressure gradient across the aortic valves was always $\leq$ 30 mm Hg.

Surgical techniques for implanting aortic and coronary artery blood flow probes, left ventricle and aortic pressure microtransducers, and coronary sinus catheters have been developed. These instruments and techniques will be used for future blood flow, blood pressure, and myocardial metabolic studies.

Summary, Part III:

Work has been initiated to study the cardiovascular responses to stressed thyrotoxic dogs. Preliminary data indicate a significant pressure gradient across the aortic valve in thyrotoxic dogs stressed by immobilization.

Progress, Part IV:

Studies designed to characterize the plasma growth hormone (PGH) response to acute infection were performed in man and the rhesus monkey. In the human studies (Project No. 69-8) basal 0800-hour PGH concentrations were determined in 13 volunteers immunized with combined Venezuelan, eastern, and western equine encephalitis vaccines (10 postinfection determinations/man) and 11 volunteers infected with sandfly fever virus (8 or 9 postinfection determinations/man). Vaccination with the trivalent encephalitis vaccine did not cause consistent elevations in PGH, nor was there any correlation between the minimal febrile responses to the vaccine and the sporadic increases in PGH concentration. In contrast, distinct elevations in PGH occurred in 10 of 11 volunteers (Project No. FY 70-1 & 70-3) infected with sandfly fever virus, (mean peak value, >10 ng/ml) with the fever preceding the initial PGH elevation in all cases. The one volunteer who failed to manifest an increase in PGH was the only subject without a febrile response to the infection.

These results, in conjunction with those obtained under similar conditions from volunteers infected with VEE vaccine, adenovirus vaccine, and typhoid fever (cumulative total of 609 control, and 1601 postinfection, PGH determinations from 87 subjects), could be interpreted as follows: Most, but not all, of the patients with acute febrile infectious illness developed increased concentrations of PGH during their acute illness. The initial rise in PGH occurred after the incubation period and usually after the onset of fever, either before or at the time of defervescence. The rarity with which increased PGH concentrations occurred in acutely ill but afebrile volunteers provides substantial evidence that the increases in PGH did not represent a psychological reaction to the stress of acute symptomatic infection. The apparent prerequisite of fever for the development of an increased PGH strongly suggests that the pathogenesis of the PGH elevation is in some manner related to that of fever. Whether or not hyperthermia in man may by itself elicit an increase in PGH, the occurrence of elevated PGH concentrations prior

to the onset of fever in at least some infected volunteers makes it unlikely that fever per se was responsible for the initial elevations. Further, the lack of correlation between 8 AM PGH values and the magnitude of fever during the preceding 8 hr suggests that fever is not the major stimulus to GH secretion during acute infection. The possibility that hypoglycemia provides such a stimulus was not supported by the occurrence of increased concentrations of both PGH and basal blood sugar levels in the 11 volunteers with acute sandfly fever. Finally, although the magnitude of the increase in PGH in patients experiencing the same acute infection tended to parallel the clinical severity of that particular illness, no such correlation existed between the PGH levels of patients with different infections. The reasons for this interesting and unexpected response of the host to different acute infections are not yet apparent.

Summary, Part IV:

Most, but not all, patients with acute febrile infections develop a modest increase in basal PGH values during their illness. The mechanisms leading to increased PGH values have not, as yet, been elicited.

Progress, Part V:

Additional studies were performed in rhesus monkeys with the basic purpose of characterizing the mechanism by which acute infection elevates PGH concentrations. Test monkeys were usually males, weighing 3-4.5 kg; they were infected at 0900 hours by the rapid IV infusion of $\geq 10^8$ viable pneumococci. Five or more days prior to infection, animals which had been adapted to isolated primate chairs underwent surgery with the insertion of an indwelling intracardiac catheter and a thermocouple positioned retroperitoneally through the lumbar paraspinal muscles. Animals studied in this manner were conscious and free of extremity restraints. They routinely responded to pneumococcal infection with a severe febrile illness of 48-72 hr duration. Pre-infection PGH values (n = 345) obtained at 2-3-hr intervals throughout the day from 21 monkeys revealed a mean concentration of 3.1 ng/ml, with a peak value of 4.9 ng/ml at 1800 hours. Although occasional animals responded to infection with increases in PGH persisting for $\geq$36 hr, consistent elevations occurred in test animals only during the 12 hr after inoculation (mean peak value, 10.7 ng/ml at 1800 hours. Similar PGH responses to infection were obtained 8 chaired, but non-isolated, monkeys bled by repeated saphenous venipuncture. In contrast to the active infection, the administration of intact, heat-killed pneumococci to non-infected animals did not elicit unequivocal increases in PGH.

Further procedures known to augment (e.g., arginine and insulin) or suppress (e.g., hyperglycemia and phenoxybenzamine) PGH under appropriate conditions were performed during the initial 12 hr of infection. Two of 3 infected monkeys given arginine (0.5 gm/kg)at 1600 hours developed additional increases in PGH (to 16.9 ng/ml), as did one of 3 infected animals given 0.1 U/kg of insulin (to 15.5 ng/ml). These results suggested

that even in severe infection with high PGH levels, GH secretion is probably not maximal. Hyperglycemia, achieved by constant glucose infusion, failed to suppress the expected PGH elevations in 3 infected animals, thereby providing further evidence that hypoglycemia is not involved in infection-induced elevations in PGH. In contrast, pre-treatment with phenoxybenzamine (1.0 mg/kg) diminished the PGH response in 3 infected monkeys (peak PGH, 5.0 ng/ml), indicating that α adrenergic receptors probably do influence the PGH response to acute infection. The nonspecificity of this finding, is apparent from the similar effect of phenoxybenzamine on PGH responses to arginine and insulin.

In nearly all monkeys with pneumococcal infection, fever preceded the initial increase in PGH. However, subsequent temperature of $\geq$ 107 F often occurred coincident with low PGH, suggesting that factors other than fever mediated the increase in PGH during infection. This observation received tentative support from recent studies which failed to demonstrate a stimulatory effect of noninfectious hyperthermia on PGH concentration in conscious animals.

The possibility that an endogenous mediator is essential to the PGH elevations of infection was investigated by administering homologous endogenous pyrogen (and intraperitoneal exudate) to conscious monkeys. Although unusually high control PGH values may well have obscured an actual response to these agents, the initial two studies (conducted at ambient temperaturesof 74 F and 82 F, respectively) failed to demonstrate unequivocal elevations not only in PGH but also in the temperature of test animals. PGH concentrations have not yet been determined in a third study (conducted at ambient temperature of 94 F) in which endogenous pyrogen elicited fever responses considered typical for this substance. Despite these inconclusive results, the possibility remains that an endogenous, humoral mediator is responsible for the PGH elevations of acute infection. This possibility is under active investigation.

Summary, Part V:

The mechanism responsible for the elevation of PGH during active infection is being investigated in the rhesus monkey.

Progress, Part VI:

There have been no prospective studies concerning metabolic fuel interrelationships during infection in man. For this reason, free fatty acids (FFA), glycerol, lactate, β-hydroxybutyrate, and glucose, as well as insulin, were measured in blood collected from 3 subjects during the course of sandfly fever study (Project No. FY 70-3). All samples were obtained at 0730 hours after $\geq$ 12 hr fasting. These results were compared with the preinfection values observed in these subjects and with the levels observed in 2 subjects who received saline instead of the virus. Since the number of subjects is small, results are discussed in terms of trends rather than statistical significance.

In the infected subjects, fasting plasma FFA levels tended to rise, attaining peak levels on the third day of infection when fever was present. Control subjects on the other hand, demonstrated fluctuation around baseline values. Fasting glycerol values tended to be slightly greater in the infected subjects on days 3 - 5 of infection, indicating the presence of increased lipolysis. The mildly elevated plasma FFA levels and slightly higher glycerol levels were not accompanied by significant changes in serum β-hydroxybutyrate or blood lactate levels.

Fasting plasma glucose levels were consistently elevated in all infected subjects on days 3 - 4 of infection when fever was present, in contrast to control subjects who displayed random fluctuations around baseline levels. Basal plasma insulin values, measured by radioimmunoassay, did not change significantly in any subject during the course of infection, most values being < 10 μU/ml, which is within the normal fasting range.

Summary, Part VI:

Sandfly fever infection is accompanied by slight increases in glucose, free fatty acid, and glycerol levels. These observations will be extended; experiments are being designed in an attempt to study the mechanisms underlying these alterations.

Presentation:

Woeber, K. A. Alterations in thyroid hormone economy during acute pneumococcal infection. Presented at American Thyroid Association, Chicago, Ill., 13-15 November 1969.

Publications:

Woeber, K. A., and W. A. Harrison. 1969. Alterations in thyroid hormone economy during acute pneumococcal infection. Program, American Thyroid Association, p. 49 (abstract).

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 003:	Tissue Enzyme Changes in Infectious Disease
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Theodore S. Herman, Major, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

10 JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 0L0802	2. DATE OF SUMMARY* 70 07 01	REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA-CONTRACTOR ACCESS [X] YES [ ] NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	003
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Tissue enzyme changes in infectious disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	2	12
	CURRENT 71	2	12

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered.

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Pneumococcus; (U) Infection; (U) Enzyme induction; (U) Tyrosine transaminase; (U) Fetal; (U) Pregnancy

23. TECHNICAL OBJECTIVE.* 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To study serial changes in tissue enzyme systems during the course of experimental infections.

24 (U) Tryptophan tolerance tests are performed serially in individuals in whom infections have been experimentally induced, in order to study the activity of hepatic tryptophan pyrrolase. Urinary diazo reactants and kynurenine pathway metabolites are measured. Since maternal and fetal tyrosine transaminase (TT) activities are sensitive to different stimuli, the pregnant female rat is used as a model system to separate different factors responsible for the rise in TT activity during infection.

24 (U) 69 07 - 70 06 - Pneumococcal infection is associated with increased TT activity in the 20- and 21-day old rat fetus but not in the 16- and 18-day old fetus. These results indicate that on days 20 and 21 increases in fetal TT cannot be explained solely on the basis of increased adrenocortical secretion alone.

Publication: Fed. Proc. 29:776, 1970 (abstract).

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 68 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 003: Tissue Enzyme Changes in Infectious Disease

Description:

To study serial changes in tissue enzyme systems during the course of experimental infection.

Progress:

Previous studies[1/] have shown that hepatic tyrosine transaminase (TT) is increased in male rats infected with Diplococcus pneumoniae organisms. This increase in enzyme activity has been attributed to an increase in the secretion of adrenocortical hormones. Hepatic TT in fetal rats has been shown to be unaffected by glucocorticoid hormones. Therefore we have used the fetal rat as our animal model in order to determine the relative importance of adrenocortical hormones in the rise in TT associated with infection.

TT was assayed in the 100,000 x g supernatant fluid of maternal and fetal livers 24 hr after the subcutaneous administration of 10^8 virulent D. pneumoniae organisms to the pregnant adults. On days 16, 18, 20 and 31 of gestation, maternal TT was increased when compared to untreated controls. D. pneumoniae could be recovered from either the liver or amniotic fluid of all of the fetuses studied. Fetal TT on days 16 and 18 of gestation, however, was unchanged. Fetal TT on days 20 and 21 of gestation was significantly increased in the infected fetuses when compared to controls. These results suggest that either pneumococcal infection on days 20 and 21 produced a change in the sensitivity of the fetal hepatocyte to glucocorticoid hormones or that a mediator other than glucocorticoid hormones played a role in the rise in TT associated with infection.

Summary:

Hepatic TT in the pregnant rat increased following pneumococcal infection just as it does in the adult male rat. Pneumococcal infection had no effect on hepatic TT in 16- and 18-day fetuses. In the 20- and 21-day old fetus, however, pneumococcal infection was associated with a significant elevation in hepatic TT activity.

Presentation:

Herman, T. S. Effect of pneumococcal infection on hepatic tyrosine transaminase (TAT) in pregnant rats and their fetuses. Presented at Federation of American Societies for Experimental Biology, Atlantic City, N. J., 12-17 April 1970.

Publication:

Herman, T. S., and W. R. Beisel. Effect of pneumococcal infection on hepatic tyrosine transaminase (TAT) in pregnant rats and their fetuses. Fed. Proc. 29:776 (abstract).

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual progress report, FY 1969. p. 15 to 23. Fort Detrick, Md.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 005:	Evaluation of Normal Colony Animals
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Animal Assessment and Pathology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Richard O. Spertzel, Major, VC (I) J. Brent Rollins, Captain, VC (I) Jerry D. Weil, Captain, VC (II) Glen E. Marrs, Jr., Captain, VC (I) Gilberto S. Trevino, Lt. Colonel, VC (III) James L. Stookey, Lt. Colonel, VC (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OLO804	2. DATE OF SUMMARY* 70 07 01		REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	005
b. [illegible]				
c. [illegible]	CDOG 1212b (9)			

11. TITLE (Precede with Security Classification Code)*
(U) Evaluation of normal colony animals

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	3	40
FISCAL YEAR CURRENT 71	3	40

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Stookey, J. L. DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Animals, laboratory; (U) Enteric infection; (U) Tuberculosis; (U) Hematology; chemistry, blood; (U) Necropsy

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Obtain clinical and pathological baseline values. Establish patterns of disease in normal colony animals.

24. (U) Conduct studies on colony animals to establish normal values for various biological parameters of interest to investigators using animals as test subjects. Study incidence and patterns of disease in the animals.

25. (U) 69 07 - 70 06 - Baseline hemograms, blood chemistry value determinations and parasitologic evaluations were done on 531 monkeys received during the year, with results similar to past years. The rhesus colony was moved from Bldg 1040 to the Phase I facility. Eleven percent of the monkeys were seropositive to staphylococcal enterotoxin B. Gastroenteritis continued to be the most common disease problem. One monkey, anergic to tuberculin, died of tuberculosis, but there were no other reactors in that group. Another group of 64 monkeys was euthanized because of numerous reactors to routine tuberculin testing. Shigella spp. was isolated from 6% of the healthy monkeys and from 42% of the monkeys affected with gastroenteritis.

The beagle colony was transferred to Walter Reed Army Institute of Research. Five hundred and forty necropsies of animals that had died spontaneously, and animals euthanized for other purposes, revealed a variety of spontaneous lesions, depending on species of animal.

Publications: J.A.V.M.A. 155:1108-1114, 1969.
Southwestern Vet. 22:99-107.
Lab. Anim. Care 1970, In press.

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 005: Evaluation of Normal Colony Animals

Description:

Obtain baseline clinical values, search for pathological lesions, and establish patterns of disease in normal colony animals.

Progress, Part I:

Data were collected on all colony animals for evaluation of normal and disease patterns.

Because of limited use, the beagle colony was transferred to the Walter Reed Army Institute of Research.

Goats and burros were screened for parasitic infestations and given anthelminthic drugs as indicated. Strongylosis was the most frequently occurring infestation encountered.

Monkeys. - A total of 531 rhesus monkeys (Macaca mulatta) were received in 6 shipments during FY 1970. Eleven percent had natural antibody against staphylococcal enterotoxin B (SEB).

By fecal examinations on 3 consecutive days (2 by $ZnSO_4$ flotation and 1 by ether-formalin concentration) nematode ova were seen in 17% of the monkeys. All parasitized monkeys were dosed with Thiabendazole, administered orally.

The collection of clinical laboratory data has continued as outlined previously.[1,2,3] Table I gives a summary of data collected during FY 1970. Portions of these data were reported at the American Association for Laboratory Animal Science.[3] The values obtained this year compare favorably with those obtained previously, Table I.

Gastroenteritis continues to be the main disease problem in the primate colony. Two groups received at Bldg. 1040 (236 monkeys) were affected with diarrhea at the rate of 35%, of which 30% were bloody. After these 2 groups subsequently were moved to Bldg. 1425, a recrudescence of gastroenteritis occurred; 30% of those not previously affected developed diarrhea within the first 30 days, whereas 55% of those previously ill developed gastroenteritis.

Four groups of monkeys have been received since moving to Bldg. 1425. In these latter 4 groups, there has been an overall diarrhea rate of 30%; 59% of the affected animals developed dysentery; Shigella spp. have been the primary bacterial pathogens that have been isolated.

TABLE I. CLINICAL LABORATORY DATA OF 1,690 SAMPLES FROM 569 MACACA MULATTA MONKEYS

VARIABLE	UNITS	MEAN ± 1 SE FY 1969[a]	MEAN ± 1 SE FY 1970
Leukocytes	no./mm^3	10,732 ± 160	12,700 ± 310
Neutrophils	%	-	32.2 ± .03
Lymphocytes	%	-	63.6 ± .32
Packed cell volume	%	40.7 ± .1	41.2 ± .1
Hemoglobin	gm/100 ml	13.3 ± .04	13.40 ± .01
Blood urea nitrogen	mg/100 ml	18.0 ± .2	19.3 ± .2
Serum transaminase glutamic oxalacetic	Sigma-Frankel	34 ± .9	32 ± .4
glutamic pyruvic	Sigma-Frankel	30 ± .5	21 ± .3
Alkaline phosphatase	Bodansky	19 ± .3	25 ± .3
Total protein	gm/100 ml	-	8.2 ± .02
Albumin	gm/100 ml	-	4.6 ± .03

a. 1,122 samples from 374 monkeys.

The move of the monkey holding facility to Bldg. 1425 allowed many improvements to be made: There are fewer monkeys per room; there is complete separation of different groups of monkeys, due to the suite arrangement; sick monkeys are put immediately into a hospital room; gloves are carboxyclaved periodically; cages and racks are disinfected every 2 weeks; exchange of room and suite equipment is restricted; and a constant-source disinfectant apparatus is in use.

We feel that nonspecific antibacterial therapy practiced prior to our receipt of the monkeys, i.e. 2 weeks' oral prophylaxis with tetracycline, compounds the problem of controlling the gastroenteritis in our monkey colony. All Shigella isolates for which antibiotic sensitivity has been determined have been resistant to tetracycline. However, the stress of movement of the animals may serve as a triggering mechanism of overt signs of gastroenteritis. During the initial months following the move, numerous fluctuations of room temperature from the desired temperature range for primates may have served as an additional stress in monkeys great enough to cause diarrhea. On one occasion, high room temperature undoubtedly contributed to a case of fatal

heat stroke and 20 other cases in which the monkeys recovered. Following a comparison of fruit-feeding versus nonfruit-feeding, the routine feeding of fruit was discontinued. Currently, fruit is fed only to anorectic monkeys.

During the past year, all monkeys were tuberculin-tested with Koch's Old Tuberculin (KOT) at 30-day intervals. One monkey, anergic to KOT, died of miliary tuberculosis, but no other reactors were found in that shipment group after 3 intrapalpebral tests, 2 weeks apart.

On 3 Nov 69, Animal Resource Section, Animal Assessment Division (AR) received 64 monkeys from the Fort Detrick Animal Farm Division. A few of the monkeys in the shipment reacted to KOT while in quarantine at the Animal Farm. The reactors were killed, and the remaining animals were retested, and found negative, on 3 occasions prior to their shipment to AR. On 24 Nov 69 all monkeys in this group were tested with KOT, intrapalpebrally. Five animals were classified as reactors and 2 as suspects. The reactors and 1 of the suspect animals were killed. All monkeys except the suspect showed gross and histologic lesions compatible with tuberculosis. The remaining monkeys were retested on 8 Dec 69. At this time, 1 new monkey reacted to KOT, and the previously identified suspect animal again reacted weakly. Both animals were necropsied. Gross and histologic lesions of tuberculosis were present in both monkeys. On the 22 Dec 69 test, 1 suspect and 55 negative reactions were observed. Two negative monkeys, selected because of their close proximity to the previous reactors, and the suspect animal, were euthanitized. No gross or histologic lesions were demonstrated.

All monkeys were tuberculin-negative on 5 Jan 70, but another reactor was detected on 19 Jan 70. Typical lesions were seen both grossly and histologically; therefore, all remaining monkeys were killed. No gross or histologic lesions compatible with tuberculosis were found in any of the 52 remaining negative reactors.

Strict quarantine was maintained in Suite 5 during this period; cages, rooms and equipment were thoroughly disinfected with a tuberculocidal agent, and equipment and cages were autoclaved when possible.

Tuberculin reactors were sporadically spaced among the 4 rooms in which the group was housed, leading us to believe that exposure occurred prior to receipt by us. Progress and control of these outbreaks were coordinated with personnel of the Fort Detrick Animal Farm Division.

All personnel associated in any way with the tuberculous monkeys had tuberculin tests and chest x-rays, with follow-up, according to Army Regulation 40-26. No tuberculin reactors or roentgenographically detectable lesions have been found, with the exception of the veterinarian-in-charge, who was positive to KOT prior to arriving at the Institute.

Six of the monkey reactors were radiographed, using intrathoracic meglumine diatrizoate to outline pulmonary lesions. Although all had gross pulmonary lesions, only 2 were detected radiographically; the contrast medium did not improve the definition of the lesions.

Minced tracheal-bronchial lymph nodes of tuberculin-reactor monkeys were cultured. No mycobacteria were isolated, even though the lesions looked very active. Mycobacterium tuberculosis was isolated from the reactor monkeys detected at the Fort Detrick Animal Farm Division.

Summary, Part I:

Clinical laboratory data were collected on rhesus monkeys. Gastroenteritis continued to be the most common disease problem. Final control of a tuberculosis outbreak required euthanasia of the 64 monkeys.

Progress, Part II:

In FY 1970 an enteric bacteriology laboratory was organized in the Phase I facility. Routine screening for Shigella in colony monkeys and diagnostic support for the colony were the functions of the laboratory. A limited research capability also is anticipated.

During November and December of 1969, while organizing the laboratory and training personnel, 339 rectal swabbings were cultured. Nineteen samples were from sick monkeys and 320 were routine samples from healthy monkeys. Shigella was isolated from 3 sick and from 17 healthy monkeys. In addition, 3 healthy monkeys shed Salmonella. Enteric pathogens were isolated from 15.8 and 6.2% of the sick and healthy monkeys, respectively.

A protocol for routine sampling of monkeys for certain bacterial enteric pathogens was developed during the first quarter of FY 1970. All animals were cultured upon receipt by Animal Assessment Division 1 and 3 weeks after arrival. Of 676 routine cultures examined, 40 were positive for Shigella for an infection rate of 5.9%. Forty sick animals were studied during the same period, 22 of which were shedding Shigella, an infection rate of 55%.

Table II summarizes the enteric pathogens isolated, the number of times each was found and whether the host was ill or healthy.

TABLE II. ENTERIC PATHOGENS OF COLONY ANIMALS

	NO. OF ISOLATIONS	
ENTERIC PATHOGEN	ROUTINE	DISEASED
Salmonella anatum	3	0
Shigella dysenteriae	6	0
Shigella flexneri 1a	2	0
Shigella flexneri 4	43	40
Shigella sonnei	6	1

Sixteen animals housed in one room of AR were extensively studied to determine the value of repeated sampling in establishing an infection rate. Rectal swabs were taken from each animal once a day, 5 days a week for 4 weeks, giving a total of 20 samples per monkey. Ten of the 16 monkeys shed Shigella at least once during this period, an infection rate of 62.5%.

Sh. flexneri 4 was identified in 7 animals, Sh. dysenteriae in 2 animals, and in the remaining animal, Sh. flexneri 4, Sh. sonnei and Sh. boydii 13 were identified.

Seven monkeys shed Shigella more than once during the study, $\leq$ 5 times. A single isolation of Shigella was made from the swab samples of 3 animals.

Summary, Part II:

An enteric laboratory was organized for routine and diagnostic support of the animal colony. In routine cultures of healthy monkeys, 60 enteric pathogens were identified from 996 cultures examined, giving an isolation rate of 6.0%. Twenty-five isolations were made from 59 cultures examined from diseased animals, an infection rate of 42.4%. In a 4-week study of 16 monkeys, 62.5% were found to shed Shigella.

Progress, Part III:

The evaluation of normal colony animals, from the pathologist's point of view, must include not only those animals that, for one reason or another, are necropsied directly from the normal colony, but all colony animals that are issued to various investigators. These animals, issued to the various investigators and necropsied by members of the Pathology Division at the conclusion of the experimental procedures, may contain not only lesions induced by the experimenter, but lesions related to various disease processes common or peculiar to the different species. These spontaneous lesions are just as prevalent in the normal colony animals before issue as they are after issue, and it is important for the pathologist, colony veterinarian and experimenter to recognize the existence of these disease processes and not to confuse them with experimentally induced lesions.

Pathology Division personnel conducted necropsies on 540 Institute animals. Of this number, only 78 were from the normal colony; of these, only 7 represented spontaneous colony deaths. The majority of the remainder of the colony animals were M. mulatta monkeys, euthanitized because of an outbreak of tuberculosis, as discussed previously in this report.

These statistics illustrate that the majority of animals necropsied by the Pathology Division represent normal colony animals, but are animals issued and utilized in experimental procedures. With this thought in mind, the remainder of this report will be a species-by-species discussion of the more common spontaneous lesions encountered in our laboratory animals.

Primates. - Several disease processes were encountered with regularity in the monkeys from our colony. Primary among them is the virtual 100% incidence of Pneumonyssus simicola, a lung mite. Another common parasite encountered is

the nematode Esophagostomum apiostomum, or nodular worm, normally found in the lower intestines. Numbers of parasites present and severity of lesions vary from animal to animal, but can be extreme. A majority of animals are affected, to varying degrees, with chronic enteritis. The incidental histological presence of small-to-moderate amounts of calcification in the adrenal cortex and ovaries is present in the majority of monkeys examined. Many cases of aspiration of antidiarrheal medication were observed. These were usually minimal-to-moderate in relation to remaining normal lung.

Rabbits. - The major disease entity encountered in our rabbits was the protozoan parasite Nosema cuniculi. Over 75% of the rabbits examined revealed lesions of the central nervous system directly related to this parasite. In addition, the incidence of chronic interstitial nephritis was high and is thought to be associated with this same parasite. The majority of livers examined contained chronic periportal hepatitis and fibrosis, the sequelae of infection with another protozoan parasite, Eimeria stiedae, commonly referred to as hepatic coccidiosis. Almost any tissue in the rabbit is susceptible to calcification following any type of insult or injury. It is common to find extensive calcification of almost any organ. Interstitial pneumonitis occurs with regularity; the primary etiology is often obscure.

Guinea Pig. - The primary deviation from the normal encountered in our guinea pigs is the diffuse infiltration of lymphoid nodules throughout the lungs. This lesion occurs in virtually 100% of our colony animals, and is often quite severe. Like the rabbit, the guinea pig is susceptible to diffuse calcification of almost any tissue.

Equine. - Several burros were necropsied during the past year and significant spontaneous lesions were encountered. Verminous arteritis, primarily of the anterior mesenteric arteries, caused by the nematode parasite Strongylus vulgaris, was observed in several animals. Histopathological examination of the kidneys revealed the presence of large numbers of the protozoan parasite Klossiella equi.

Other animal species examined by the Pathology Division included the dog, pig, goat, rat, mouse and gerbil. No pertinent comments are made concerning these species, either because of lack of numbers necropsied, or the absence of significant spontaneous lesions.

Summary, Part III:

Five hundred and forty necropsies of animals that had died spontaneously, and animals euthanitized for other purposes, revealed a variety of spontaneous lesions, depending on species of animal.

Presentations:

1. Rollins, J. B., C. H. Hobbs, R. O. Spertzel, and S. McConnell. Hematologic studies of the rhesus monkey (Macaca mulatta). Presented at American Association for Laboratory Animal Science, Dallas, Texas. 13-17 October 1969.

2. Weil, J. D., H. S. Kessler, M. K. Ward, and R. O. Spertzel. Incidence of Shigella species in clinically healthy monkeys (Macaca mulatta). Presented at American Association for Laboratory Animal Science, Dallas, Texas. 13-17 October 1969.

Publications:

1. Koller, L. D.: 1969. Spontaneous Nosema cuniculi infection in laboratory rabbits. J. A. V. M. A. 155:1108-1114.

2. McConnell, S., H. W. Whitford, R. D. Feigin, R. D. Harting, and R. A. Vandercook. 1969. Use of a plastic window to obtain serial liver, spleen and kidney biopsies in the rhesus monkey. Southwestern Vet. 22:99-107.

3. Rollins, J. B., C. H. Hobbs, R. O. Spertzel, and S. McConnell. 1970. Hematologic studies of the rhesus monkey (Macaca mulatta). Lab. Anim. Care. In press.

LITERATURE CITED

1. U. S. Army Medical Unit. 1 July 1968. Annual Progress Report, FY 1968. p. 29 to 33. Fort Detrick, Maryland.

2. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual Progress Report, FY 1969. p. 25 to 30. Fort Detrick, Maryland.

3. Rollins, J. B., C. H. Hobbs, R. O. Spertzel, and S. McConnell. 1970. Hematologic studies of the rhesus monkey (Macaca mulatta). Lab. Anim. Care. In press.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 008:	Cellular Changes During the Immune Response
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Harry G. Dangerfield, Lt Colonel, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

32

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0869	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	K. COMPLETION	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	008
b. ~~CONTRIBUTING~~				
c. ~~CONTRIBUTING~~	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Cellular changes during the immune response

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 08	70 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: NA d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	0	0
FISCAL YEAR CURRENT 71	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, Md 21701

RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-4111 Ext 5233

20. PERFORMING ORGANIZATION

NAME: Bacteriology Division
USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, Md 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Dangerfield, H. G.
TELEPHONE: 301 663-4111 Ext 7341
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign intelligence considered.

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Immunity; (U) Immunology; (U) RNA; (U) DNA; (U) Antibody formation

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate biochemical changes in individual antibody-producing cells during the immune response, with emphasis on quantitative and qualitative changes in nucleic acid metabolism.

24 (U) Suspensions of lymphoid cells are prepared from immunized animals. Specific antibody-producing cells are identified by means of the antibody plaque technique. DNA, RNA, and protein synthesis are then studied by means of autoradiography.

25 (U) 69 07 - 70 06 - No work has been performed during the current fiscal year. The publication completes this work unit.

Publication: Nature 222:1295-1296, 1969.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 008: Cellular Changes During the Immune Response

Description:

Investigate biochemical changes in individual antibody-producing cells during the immune response, with emphasis on quantitative and qualitative changes in nucleic acid metabolism.

Progress and Summary:

No work has been performed during the current fiscal year. The publication shown completes this work unit.

Publications:

1. Krisch, R. E. 1969. DNA synthesis by antibody-forming cells during the primary immune response. Nature 222:1295-1296.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 01:	**Pathogenesis of Infection of Military Importance**
Work Unit No. 096 01 009:	**Amino Acid and Protein Changes in Blood in Infectious Disease**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Physical Sciences, Medical and Animal Assessment
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Robert W. Wannemacher, Jr., Ph.D. (I, II) William R. Beisel, M.D. (I-III) George A. Burghen, Major, MC (III) J. Brent Rollins, Captain, VC (III) Jerry S. Walker, Major, VC (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0L0879	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	009
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Amino acid and protein changes in blood in infectious disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	3	40
FISCAL YEAR CURRENT 71	3	40

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, Md 21701

RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-4111 Ext 5233

20. PERFORMING ORGANIZATION

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USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, Md 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Wannemacher, R. W.
TELEPHONE: 301 663-4111 Ext 6130
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Beisel, W. R.
NAME: Vollmer, R. T. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Amino acids; (U) Sandfly fever; (U) Monkeys; (U) Pneumococcus

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study changes in amino acids of blood and tissues in infectious disease or in conditions induced by other variables.

24 (U) Free amino acid concentrations are determined by ion-exchange chromatography in plasma and tissue of experimental subjects infected with bacterial or viral organisms.

25 (U) 69 07 - 70 06 - Studies to determine free amino acid changes during the course of infection with various microorganisms in man and laboratory animals have been continued.

Both viral and bacterial infections in human and nonhuman models are characterized by an early depression in plasma free amino acids. This drop is correlated with the elevated rate of protein synthesis in visceral tissues. As rats approach terminal states of infection, muscle tissue is broken down to supply amino acid for the elevated rate of protein synthesis and metabolism in visceral tissues such as liver. The non-protein synthetic pathways for certain amino acids are also altered by infection and these changes are reflected in the tissue levels of these amino acids.

Specifications were developed for an automated data acquisition system for the Institute. Fortran IV programs were written to automate partially data reduction and to initiate a scientific data file. Study was undertaken to develop mathematical models appropriate to biological parameters which reflect presence of infection.

Publication: Arch. Intern. Med. 123:620-625, 1969.
Fed. Proc. 29:820, 1970 (abstract).
Amer. J. Clin. Nutrition 23:660, 1970

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 009: Amino Acid and Protein Changes in Blood in Infectious Disease

Description:

Study changes in amino acids of blood and tissues in infectious disease or conditions induced by other pertinent variables and infection-related changes in plasma protein fractions.

Progress, Part I:

Earlier data obtained by paper chromatographic separations of whole blood amino acids revealed that both bacterial and viral infections in man resulted in a drop in total blood amino acids. Volunteers were infected with either Pasteurella tularensis,[1] Salmonella typhosa,[2] live attenuated Venezuelan equine encephalomyelitis,[3] or with the 17D vaccine strain of yellow fever[4,5] and total blood amino acid concentrations were measured serially during the course of the infection. From these data it may be concluded that the magnitude and length of time that the blood amino acids are depressed is correlated with the degree and duration of the febrile response.

A similar study was undertaken in 10 volunteers (Project No. FY70-1), 8 of whom were infected with sandfly fever virus, with the exception that plasma free amino acids were separated by ion-exchange chromatography; there were 2 controls. Sandfly fever is a benign viral infection which is characterized by onset of fever in 60-70 hr, a cessation of fever 36-48 hr after onset, leukopenia, depression of serum Zn and Fe and a rise in serum Cu. By 48 hr, the total plasma free amino acids were depressed to 50-60% of pre-exposure control values. These changes in plasma amino acids occurred before the onset of fever or any other clinical indications of illness and did not occur in control subjects. As the febrile response progressed the total plasma amino acids were depressed to 30% of control values. The plasma levels of those amino acids which have been reported to be essential for protein synthesis, and especially leucine, were reduced to greater degree than those which enter mainly glucogenic or ketogenic pathways. During the first 24 hr after exposure to the virus, food intake was normal and the excretion of urinary urea and amino nitrogen was not altered. Thus, the marked depression of plasma amino acid during the prodromal stages of infection could not result from an increased rate of catabolism and excretion of amino acids or from protein deprivation. As the infection progressed, food intake was reduced but the magnitude of the drop in plasma amino acids was far greater than that observed during mild starvation in man.

A computer program has been developed for the statistical analysis of the changes in individual amino acids as well as for grouping of metabolically similar acids. Individual subjects have been compared to their own pre-infected control values and to the changes within an experimental group.

A similar rapid fall in plasma amino acid was observed in monkeys following a systemic challenge with virulent Diplococcus pneumoniae, type A-1, strain 5. However, in this infection there was a transient (15-150 min), rise in the concentration of total plasma free amino acids. By 3 hr postchallenge all of the plasma free amino acids were reduced below pre-infection values. The total plasma amino acid level remained low for the next 96 hr. While the decrease in plasma amino acid preceded the rise in body temperature, the plasma Zn and Fe were significantly decreased at 15 min. During the early stages of the infection the major decrease was in those amino acids that are utilized principally for protein synthesis, especially leucine, while during the later phases the amino acids that can enter the glucogenic and ketogenic pathways accounted for the depression in plasma free amino acid concentrations.

When monkeys were challenged with a systemic infection of Salmonella typhimurium, the plasma free amino acids were markedly depressed at the end of 8 hr but were at near normal values 96 hr postinfection, even though the febrile response lasted for 22 days. The response to this infection is currently under investigation in the monkey model and is being compared to a new human study with S. typhosa (in collaboration with the contractor, Dr. Richard B. Hornick, University of Maryland, Contract no. DA 49-193-MD-2867).

Summary, Part I:

Both viral and bacterial infections in human and nonhuman models are characterized by an early depression in plasma free amino acids. The major drop during the prodromal phases of the infection is not related to decreased food intake or increased nitrogen loss, but is correlated with a decrease in amino acids that enter protein synthetic pathways. In later stages of infection when there may be an increase in nitrogen loss, the amino acids which enter glucogenic and ketogenic pathways account for the major depletion in plasma free amino acid content.

Progress, Part II:

In order to understand the significance of the infection-induced depression of plasma amino acids, it was necessary to measure the tissue free amino acid levels in an infection in an animal model. The model chosen was the subcutaneous infection of D. pneumoniae, type I-A, strain 5, in Fisher-Dunning rats. An acute pneumococcal infection in the rat is characterized by an initial rise in body temperature 8-14 hr postinfection, a maximal increase in body temperature at 24-30 hr, and a subsequent decrease to subnormal temperature and death in 55-70 hr. Bacteremia was observed in 1 of 4 animals at 14 hr and was present in increasing amounts in all animals as the infection progressed. The rats

developed leukopenia, first in lymphocytes than neutrophils; on days 1 and 2, food intake was reduced to 30 and 10%, respectively, of pre-exposure values. Thus, in the subsequent experiments food was removed from both saline control and infected animals at 24 hr postexposure. Both saline controls and infected animals were necropsied at various times after exposure; protein-free filtrates of serum, liver and skeletal muscle were analyzed for their amino acid content by ion-exchange chromatography. During the first 24 hr postinfection, free amino acids in serum, liver and muscle of the infected rats tended to decrease while those in the saline controls showed a typical circadian periodicity with highs in the morning and lows in the evening. At the end of 24 hr the free amino acid content of all tissues studied in the infected animals was 60-65% of that found in comparable controls. Earlier data from our laboratory had indicated that protein synthesis is markedly elevated in the liver and depressed in muscles of animals that have been infected with virulent D. pneumoniae for 24 hr.[6] From these observations it may be concluded that amino acids are being shunted from skeletal muscle to viscera where they are being rapidly synthesized into proteins. As the infection progressed, the levels of free amino acids increased in serum, liver and muscle; in fasted saline controls, liver and serum values still exhibited circadian periodicity and skeletal muscle levels were markedly reduced. The latter increase in muscle free amino acid of infected rats correlated with the increased catabolism that has been observed during terminal stages of infection.[7] The increase in liver and serum amino acids may be related to a reduced rate of liver protein synthesis that has also been seen during the late phases of pneumococcus infection in animals.[6]

When individual amino acids were compared during early (4 hr), active (24 hr), and late (48 hr) stages of infection, it was noted that most of the amino acids essential for protein synthesis responded in a similar manner. There is a gradual decrease in these serum, liver, and muscle amino acids during the first 24 hr and a rise in serum and muscle concentrations during the second 24 hr. Generally, an amino acid which could enter other metabolic pathways had some deviation from this pattern of protein-essential amino acids. Responses of phenylalanine and arginine were particularly unusual. The phenylalanine values were elevated in serum and skeletal muscle at 24 and 48 hr while the liver concentrations were low. Free arginine values were usually low in liver, but at 24 and 48 hr there was a significant increase in the liver concentration of this amino acid when compared to controls.

The levels of alanine, the major glucogenic amino acid, in liver and muscle were low 24 hr postexposure while 24 hr later they were very high. These results suggest that glucogenesis was high during active infection but was reduced during terminal stages of the disease. This would agree with the earlier hyperglycemia and later hypoglycemia in this infectious model (See Work Unit 096 01 002).

The muscle levels of glutamic acid and glutamine (the major ketogenic amino acids) were below control levels at 24 and 48 hr, suggesting that they were being utilized as a source of energy.

The serum and muscle levels of tyrosine were low at 24 and 48 hr, while the phenylalanine: tyrosine ratio was very high as compared to saline controls. These findings are in agreement with reports of Newberne and co-workers[8] that both viral and bacterial infections in dog and rat result in an elevated serum phenylalanine: tyrosine ratio.

Summary, Part II:

In rats infected with pneumococci the drop in plasma amino acids correlates with the elevated rate of protein synthesis in visceral tissues. As the animals approach terminal stages of infection, muscle tissue is broken down to supply amino acid for the increased rate of protein synthesis and metabolism in visceral tissues, such as liver. The nonprotein synthetic pathways for certain amino acids were also altered by infection as reflected by the tissue levels of these amino acids.

Progress, Part III:

Subsequent to the development of a rapid and reliable method for differentiating glycoprotein fractions of serum,[9] a number of studies were conducted in volunteers during the past several years to quantitate serial changes in tularemia, sandfly fever, Rocky Mountain spotted fever (in a cooperative study with Dr. Richard Hornick at the University of Maryland), and following vaccination with 17-D strain of yellow fever virus, Live Attenuated VEE virus vaccine; and Live Attenuated Oral Type 7 Adenovirus. Serial blood samples were also obtained by Dr. Albert S. Klainer at Ohio State University (Contract No. DA DA 17-68-C-8080) from patients during a variety of naturally-acquired infections. All these data have been subjected to statistical analysis and are being prepared for publication.

Two additional studies have been completed in dogs. In the first study young beagle dogs, some immune and some not immune to infectious canine hepatitis, were given either a virulent or attenuated strain of infectious canine hepatitis virus (ICH). Nonimmune dogs infected with virulent ICH virus showed large early increases in α_2 glycoglobulin which occurred prior to hepatic enzyme increases and hepatocellular damage. Immune dogs given attenuated or virulent ICH virus and nonimmune dogs given attenuated virus showed no such changes.

These observations imply that neither the entry or replication of a virus in the host nor cellular destruction serves to stimulate the early α_2 glycoglobulin increase. Rather, this phenomenon appears to be a nonspecific host response associated with the onset of systemic illness. This material has been prepared for publication and is undergoing editorial evaluation.

The second study in dogs was accomplished as a cooperative effort with COL R. M. Nims, VC, Major D. L. Huxsoll, VC, and Major P. K. Hildebrandt, VC, of the Division of Veterinary Medicine, Walter Reed Army Institute of Research. Initial serum samples for this study originated in Vietnam and were acquired as part of an investigation of a fatal disease in U. S. military dogs. It was found that dogs in the terminal pancytopenic or epistaxis stage of naturally acquired tropical canine pancytopenia (TCP) developed serum alterations characterized by hypergammaglobulinemia, hypergammaglycoglobulinemia, and a concomitant decrease in serum albumin. The β globulin and glycoglobulin fractions were increased slightly while the α fractions were either decreased or normal. When transmitted experimentally in puppies, the febrile phase of the initial illness was accompanied by a transient slight rise in the α_2 protein and glycoprotein fractions. This was followed in turn by a prolonged period in which values of the γ globulin and glycoglobulin fractions rose progressively.

The magnitude of increase in the γ protein and glycoprotein fractions correlated with duration of illness. By the time dogs first exhibited epistaxis they had already entered into an advanced phase of the disease. The data could be interpreted to suggest occurrence of a possible hypersensitivity reaction or an autoimmune response during the chronic phase of TCP. This material has been prepared for publication and is now in the process of being evaluated for clearance.

Summary, Part III:

A wide variety of studies have been conducted in man and experimental animals to gain an understanding of the timing, magnitude, and nature of the appearance of acute phase protein reactants in serum during a variety of infectious illnesses.

Presentation:

Wannemacher, Jr., R. W. Significance of changes in plasma free amino acids. Presented at Federation of American Societies for Experimental Biology, Atlantic City, N. J. 12-17 April 1970.

Publications:

1. Klainer, A. S., P. F. Gilliland, W. J. Cirksena, P. J. Bartelloni, and W. R. Beisel. 1969. Serum glycoproteins in naturally acquired malaria in man. Arch. Intern. Med. 123:620-625.

2. Wannemacher, Jr., R. W., R. E. Dinterman, and W. R. Beisel. 1970. Significance of changes in plasma free amino acids. Fed. Proc. 29:820 (Abstract).

3. Burghen, G. A., W. R. Beisel, and P. J. Bartelloni. 1970. Influences of chloramphenicol administration on whole blood amino acids in man. Clin. Med. In press.

4. Beisel, W. R., R. W. Wannemacher, Jr., R. S. Pekarek, and P. J. Bartelloni. 1970. Early changes in individual serum amino acids and trace metals during a benign viral illness of man. Amer. J. Clin. Nutr. 23:660 (Abstract).

LITERATURE CITED

1. Feigin, R. D., and H. G. Dangerfield. 1967. Whole blood amino acid changes following respiratory-acquired Pasteurella tularensis infection in man. J. Infect. Dis. 117:346-351.

2. Feigin, R. D., A. S. Klainer, W. R. Beisel, and R. B. Hornick. 1968. Whole blood amino acid in experimentally-induced typhoid fever in man. New Engl. J. Med. 278:293-298.

3. Feigin, R. D., R. F. Jaeger, R. W. McKinney, and A. C. Alevizatos. 1967. Live attenuated VEE virus vaccine. II. Whole blood amino acid and fluorescent antibody studies following immunization. Amer. J. Trop. Med. 16:769-777.

4. U. S. Army Medical Unit. 1 July 1968. Annual progress report, FY 1968. p. 36 to 44. Fort Detrick, Maryland.

5. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual progress report, FY 1969. p. 37 to 44. Fort Detrick, Maryland.

6. Lust, G. 1966. Effect of infection on protein and nucleic acid synthesis in mammalian organ and tissues. Fed. Proc. 25:1688-1694.

7. Beisel, W. R., W. D. Sawyer, E. D. Ryll, and D. Crozier. 1967. Metabolic effects of intracellular infections in man. Ann. Intern. Med. 67:744-779.

8. Newberne, P. M., V. R. Young, and J. F. Gravlee. 1969. Effects of caloric intake and infection on some aspects of protein metabolism in dogs. Brit. J. Exp. Path. 50:172-180.

9. Klainer, A. S., W. R. Beisel, and W. K. Atkins. 1968. Determination of serum glycoproteins on cleared cellulose acetate strips. Amer. J. Clin. Path. 38:137-141.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 01:	**Pathogenesis of Infection of Military Importance**
Work Unit No. 096 01 010:	**Effect of Irradiation on Infection and Immunity**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Animal Assessment and Medical
Period Covered by Report:	1 July 1969 through 30 June 1970
Professional Authors:	Richard O. Spertzel, Major, VC John C. Holder, Major, MC Douglas W. Mason, Captain, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0883	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	010
b. CONTRIBUTING	//////			
c. CONTRIBUTING	//////	CDOG 1212b(9)		

11. TITLE (Precede with Security Classification Code)

(U) Effect of irradiation on infection and immunity

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	0	0
FISCAL YEAR CURRENT 71	1	10

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, Md 21701

RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-4111 Ext 5233

20. PERFORMING ORGANIZATION

NAME: Animal Assessment Division
USA Medical Research Institute of Infectious Diseases
ADDRESS: Fort Detrick, Md 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Spertzel, R. O.
TELEPHONE: 301 663-4111 Ext 7244
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Holder, J. C.
NAME: Mason, D. W. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede each with Security Classification Code)

(U) Irradiation; (U) Immunity; (U) Infectious diseases; (U) Encephalitis, equine (VEE); (U) Yellow fever

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate interrelationships between acute or chronic irradiation and disease processes.

24 (U) Acute or protracted whole body irradiation is delivered to selected animal species before, simultaneously with, or after infection. Clinical and immune responses are observed and measured serially.

25 (U) 69 07 - 70 06 - The MEV radiation source is installed in the medical laboratory and is awaiting final Health Physics inspection. The cobalt source for chronic irradiation has not been released to the Institute by the contractor.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 010: Effect of Irradiation on Infection and Immunity

Description:

Investigate interrelationships between acute and chronic irradiation and disease processes.

Progress and Summary:

The one ME-V irradiation source has been installed in the new medical laboratory and is awaiting final Health Physics inspection. The cobalt source for chronic irradiation has not been released to the Institute by the contractor.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 011:	Biophysical Studies of Pathogenic Microorganisms
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Anne Buzzell, Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0810	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	011
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212B(9)			

11. TITLE (Precede with Security Classification Code)
(U) Biophysical studies of pathogenic microorganisms

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 02	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	20
FISCAL YEAR CURRENT 71	1	10

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: 301 663-4111 Ext 6237
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Quin S.
NAME: DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Virus; (U) Electron microscopy; (U) Negative staining

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To develop a rapid electron microscopic assay for virus particles.

24 (U) A method is being developed which involves the transfer of virus from a millipore filter to an electron microscope grid with negative staining.

25 (U) 69 07 - 70 06 - The problems, which had been encountered in the step of the electron microscopic assay for virus particles in which virus is transferred from a millipore filter to an electron microscope grid with negative staining appear to have been the result of chemical interaction between the filter and negative stain, sodium phosphotungstate. Means of avoiding the difficulty appear to have been worked out so that the new procedures can now be tested for improving the centrifugation step to increase the sensitivity and rapidity of the assay. However, this work was postponed while a literature survey was being made in connection with the theoretical model for the biological membrane described elsewhere in this report.

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 011: Biophysical Studies of Pathogenic Microorganisms

Description:

To develop a rapid electron microscopic assay for virus particles.

Progress:

As reported last year[1], a rapid electron microscopic assay for virus particles in unpurified dilute suspensions is under development. The assay is derived from that of Sharp[2] in which virus was first concentrated by centrifuging down onto a piece of agar. As described earlier, a much simpler procedure for transferring the virus from agar to the electron microscope grid has been evolved which incorporates negative staining, thus facilitating identification of the virus. Furthermore, the new procedure is not limited, as was Sharp's, to transfer from agar. As reported last year troubles were encountered in controlling the flow of negative stain solution through millipore filters and chemically related material, but a survey of the literature indicated the probable source of the problem was chemical interaction between the filter and the negative stain, sodium phosphotungstate. Enough experiments have since been done to indicate that the problem was correctly diagnosed, and that quantitative transfer of virus from millipore filters with negative staining should be possible and that the ideas, described in previous reports, for modifying the centrifugation step can be tried which should greatly increase the sensitivity of the assay. Further experiments were postponed pending completion of the literature survey being made in connection with the theoretical model being worked out for the biological membrane which is described elsewhere in this report. Since much of the literature being studied concerns surface phenomena and transport of ions through small pores, it was felt that the literature survey would probably prove useful for the virus assay project too.

Summary:

The problems encountered in the step of the virus assay in which virus is transferred from a millipore filter with negative staining appear to have been solved. Thus, the new centrifuge procedure for greatly increasing the sensitivity of the assay can now be tried. However this work has been postponed because of the literature survey being made in connection with the new theoretical model of the biological membrane described elsewhere in this report (Work Unit 096 01 012).

Publications:

None

LITERATURE CITED

1. U. S. Army Medical Unit. 1 July 1969. Annual Progress Report, FY 1969, p. 51 to 54. Fort Detrick, Maryland.

2. Sharp, D. C. 1949. Enumeration of virus particles by electron micrography. Proc. Soc. Exp. Biol. Med. 70:54 to 59.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 01:	**Pathogenesis of Infection of Military Importance**
Work Unit No. 096 01 012:	**Biophysical Studies of Bacterial Toxins and Other Inert Molecules**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Anne Buzzell, Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

50

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION* DA OL0889	2. DATE OF SUMMARY* 70 07 01	REPORT CONTROL SYMBOL DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	012
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Biophysical studies of bacterial toxins and other inert molecules

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, BW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	30
FISCAL YEAR CURRENT 71	2	30

19. RESPONSIBLE DOD ORGANIZATION
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NAME:* Buzzell, A.
TELEPHONE: 301 663-4111 Ext 6237
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered.

DA

22. KEYWORDS (Precede each with Security Classification Code)
(U) Latex particles; (U) Membrane; (U) Pore; (U) Phospholipid; (U) Ion; (U) Chelate; (U) Ultracentrifugation; (U) Micelle

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Characterize various toxins or other inert molecules which have biologic activity.

24 (U) Ultracentrifugation will be used to study the interactions of toxins and other inert molecules with the host tissues.

25 (U) 69 07 - 70 06 - Improvements in the technique for determining density of solutions in the ultracentrifuge from the sedimentation rate of large latex spheres allow density differences to be measured down to 1 μg/ml. Density variations of the latex particles revealed that the alkyl sulfonate micellar layer which coats the particle was a strong and selective chelator of divalent cations. This suggested that the cation selective pores of the external membranes of mammalian cells might be similar micelles of fatty acid soaps. Since the principal membrane phospholipids should be able to form linear micelles, they would provide strands to surround soap micelles and other material. A detailed analysis of the characteristics to be expected for linear micelles of phosphatidic acid, lecithin and phosphatidyl inositol indicated that a membrane of this nature could account not only for ion transport but for transport of amino acids and sugars as well.

Publication: Biophysical Society Abstracts 10:42a, 1970.

*Available to contractors upon originator's approval.

DD FORM 1498 1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 012: Biophysical Studies of Bacterial Toxins and Other Inert Molecules

Description:

Characterize various toxins or other inert molecules which have biological activity.

Progress:

During the biophysical study of components of anthrax toxin[1] resolved by sucrose density gradient centrifugation the need arose for a method of measuring partial specific volumes of materials in sucrose solutions. In previous reports[2,3] a simple method was described that involved sedimenting large spherical latex particles in sucrose solutions with density adjusted close to that of the latex particles. A means of eliminating the need for precise temperature control was discovered which made it possible to measure density differences down to 1 μg/ml. As the density measurements became more sensitive, variations in the latex particle density became apparent which revealed some interesting characteristics of the alkyl sulfonate micellan layer which coats the particles. The principal finding was that the sulfonate micelle was a strong and selective chelator of alkaline earth cations. Each latex particle could bind 16 x 10^6 Ba^{++} or Mg^{++} ions, apparently by bidentate chelation, suggesting the sulfonate molecules formed hexagonal array which would allow a barium ion, or a hydrated magnesium ion, to fit between sulfonates across a hexagon. The sulfonate molecules appear free to shift (as is known to occur in free detergent films) to form a square array allowing tetradentate chelation of calcium, since only 8 million Ca^{++} ions are found per latex particle and binding is stronger than for Mg or Ba. When this work was reported at an International meeting in September 1969, suggestion was made that similar micelles, for instance ones of fatty acid soaps might provide pores in external membranes of mammalian cells, which must be able to admit K^+ ions but exclude Ca^{++} ions. This basic idea has since been expanded and has culminated in a unitary model of the biological membrane which appears to account not only for transport of ions into cells but also for transport of amino acids and probably sugars as well. The salient features of the model are as follows.

It appeared quite possible that soap micelles might be embedded in a phospholipid framework, when analysis showed that all the principal phospholipids should form linear micelles, providing strands to surround soap micelles and other material. Phosphatidic acid micelles, which for a number of reasons seemed most likely to trap soap micelles, could form zipper-like structures which could close by chelating Ca^{++} ions between neighboring phosphate groups. With proper compression the trapped soap molecules would chelate Ca preventing entry into the cell. K^{+} ions should also fit down between the carboxyl groups reducing the charge on the outer surface of the soap micelle. However the monovalent cations could dissociate readily, and those moving inward would be pulled across by the higher charge on the inside surface of the soap micelle acting through a region of low dielectric constant. Further compression of the soap to allow the smaller Na^{+} ion to be sequestered and transported, could be produced by an ATPase enzyme, embedded near the micelle, if the enzyme were expanded by entrance of an ATP molecule. The model thus accounts for the "sodium pump" action of ATPase. A phosphatidic acid zipper could also account for Lowenstein's finding[4] that where cells join, external membranes become porous, but revert back to normal if exposed to Ca^{++} ions.

Neutral (and basic) amino acids should enter the soap micelle to remove the hydrophobic portion out of water. Near the micelle surface pH can be quite low, since cations cluster near a strong negative charge. Therefore amino acids accumulating in the micelle should occasionally turn cationic and cross the membrane when the charge on the inner surface is greater. Sodium ions, which aid transport of amino acids, would fit between carboxyls when the soap was crowded enough by the accumulating amino acid molecules and thus lower the charge on the outer surface of the micelle.

Linear micelles of lecithin should provide both an anchor for the zipper, which is indpendent of Ca^{++} ions, and also pores to admit anions into the cell. The phosphate residues of this choline ester of phosphatidic acid being monobasic, alternate phosphates in the micelle could be maintained uncharged if they were hydrogen bonded to neighboring charged phosphate residues. With the quaternary amines of the choline residue out at the sides of the linear micelle, the strand edged by positive charges could anchor the negative charges on a phosphatidic acid strand. Lecithin strands could be held together by cholesterol, which is known to make lecithin films more compact. Since oxygens from the neutral phosphate groups would point toward grooves in the micelle, between the protruding amines of neighboring lecithin molecules, the hydroxyl residue of cholesterol, which is known to be essential for its activity, might form a hydrogen bond to the phosphate. Grooves near the charged phosphate residues would be less likely to accomodate cholesterol. Holes therefore would be left open, and being rimmed by amines, should attract and allow passage of amines into the cell. Water probably would enter by this route since entry of water into cells can be blocked by large anions. Other phospholids might provide for sugar transport, making pores rimmed by inositol or other hydrophobic groups; for Na dependent sugar transport, which is common, the pores could be rimmed by amines at the inner surface. Then, in

the absence of Na^+ ions, anions might nestle in the sugar pores, blocking them off. The polypeptide insulin, therefore, may enhance the Na dependent transport of sugars by wedging into an adjacent soap micelle. The crowded soap would sequester and transport Na inside to attract the anions out of the sugar pores. A survey of the literature, now being made suggests that the membrane model will be applicable to membranes of many different types, functional differences being accounted for by variations of the general features described above.

Summary:

A unitary model of the biological membrane has been worked out, the principle feature being inclusion of soap micelles in a framework of linear micelles of phospholipids such as phosphatidic acid, lecithin, and phosphatidyl inositol. It was shown that such a membrane could account for transport not only of ions, but of amino acids and sugars as well.

Presentations:

1. Buzzell, A. Ultracentrifugal analysis of metal chelation by polyvinyl toluene latex spheres: Application of the phenomenon to biological membranes. Presented at 3rd International Biophysics Congress of the International Union for Pure and Applied Physics, Cambridge, Mass. 29 Aug-3 Sep 1969.

2. Buzzell, A. Metal chelation by detergent micelles: A model for the small pores of biological membranes. Presented at 14th Annual Meeting of Biophysical Society, Baltimore, Md. 25-27 February 1970.

Publications:

1. Buzzell, A. 1970. Metal chelation by detergent micelles: A model for the small pores of biological membranes. Biophysical Society Abstracts 10: 42a (abstract).

LITERATURE CITED

1. Buzzell, A. 1967. Structural model for the lethal components of anthrax toxin based on ultracentrifuge studies. Fed. Proc. 26: 1522 to 1526.

2. U. S. Army Medical Unit. 1 July 1968. Annual Progress Report FY 1968. p. 57 to 60. Fort Detrick, Maryland.

3. U. S. Army Medical Unit. 1 July 1969. Annual Progress Report FY 1969. p. 55 to 58. Fort Detrick, Maryland

4. Lowenstein, W. R. 1967. On the genesis of cellular communication. Developmental Biol. 15:503 to 520.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 013:	Host Lipids in Infectious and Toxic Illness
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Physical Sciences, Medical, and Animal Assessment
Professional Authors:	Robert H. Fiser, Jr., Captain, MC (I-III) William R. Beisel, M.D. (I) George H. Burghen, Major, MC (I) Robin T. Vollmer, Captain, MC (I) Joseph C. Denniston, Jr., Captain, VC (III) J. Brent Rollins, Captain, VC (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OLO900	70 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 05 28	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	013
b. [illegible]				
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Host lipids in infectious and toxic illness

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: NA d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	2	10
	CURRENT 71	2	10

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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TELEPHONE: 301 663-4111 Ext 5158

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Denniston, Jr. J. C.

NAME: Beisel, W. R. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Lipids; (U) Bacterial infections; (U) Viral infections; (U) Enterotoxemia; (U) Endotoxin

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study early changes in lipid metabolism during infectious and toxic illness.

24 (U) Measure serum lipids and lipoproteins during induced illness and examine the kinetics of fatty acid metabolism.

25 (U) 69 07 - 70 06 - In volunteers infected with sandfly fever virus there is a decrease in serum cholesterol and triglycerides which begins within the first 24 hr after infection and is more marked at the height of the febrile state. There is a decrease in the pre-beta lipoprotein fraction and an increase in the alpha fraction at similar times. The latter fraction begins to fall during the 7th to 8th day of infection and returns to normal values during convalescence. Ultracentrifugation studies show a decrease in the cholesterol content of beta fraction, most marked on the 3rd and 4th day of illness.

Monkeys intoxicated with staphylococcal enterotoxin B and endotoxin show increased utilization of fatty acids and incorporation of labeled fatty acid into the triglyceride fraction in the liver.

Beagle dogs given a high fat diet show a decreased resistance to infectious canine hepatitis. The reasons remain unclear. Lipoprotein electrophoretic patterns show changes compatible with an increased rate of lipolysis at the height of the illness. Ponies and burros immunized with live Venezuelan equine encephalomyelitis vaccine have no significant changes in their lipoprotein levels.

Publication: Amer. J. Clin. Nutr. 1970. In press.

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 013: Host Lipids in Infectious and Toxic Illness

Description:

Study early changes in lipid metabolism during infectious and toxic illness.

Progress, Part I:

Since lipid metabolism is intimately related to energy requirements of the host and subsequent body defenses, studies were begun in an attempt to understand the mechanisms involved in lipid alteration during an infectious illness. Few details are known concerning the influences of an infectious illness on body lipids; however, recent studies[1] have served to indicate that body fat is mobilized during the infectious process. These studies show that body lipids may respond in a somewhat different manner according to the etiologic agent involved.

It seemed desirable to study serum lipoprotein changes in conjunction with those of serum glycoproteins and proteins (the latter are reported in Work Unit No. 096 01 009). A rapid and reliable method was standardized for the electrophoretic separation and densitometric quantitation of serum and plasma lipoproteins.[2] This method employs cellulose polyacetate strips, staining with Schiff reagent after a 10-min exposure to ozone, and complete clearing of background opacity. The β, pre-β and α lipoprotein bands are easily quantitated by this procedure as are any dietary chylomicrons present. Normal values were documented for 70 healthy adult male volunteers (Project No. FY 69-9) along with data on day-to-day variability of individual subjects. Changes following the ingestion of high fat or carbohydrate meals, or the lipoprotein response to an acute stress (febrile vaccine reaction) were studied serially. Optimal conditions for the storage of samples were defined. The method appears useful for screening large numbers of patients or for side-by-side comparison with protein and glycoprotein electrophoretic studies of the same sample.

A complete review of the literature was carried out to ascertain known interrelationships between lipid metabolism and infectious illness. This information is soon to be published as a "Perspective in Nutrition" in the American Journal of Clinical Nutrition.

Since there had been no prospective studies of lipid metabolism during an infectious illness in man, a small sandfly fever study in 3 volunteers (Project No. FY 70-3) was carried out as part of a cooperative study with Dr. Robert S. Lees, Massachusetts Institute of Technology, in an attempt to obtain

TABLE I. PLASMA CHOLESTEROL, QUANTITATIVE LIPOPROTEINS[a] (DERIVED BY ULTRACENTRIFUGATION[b]) AND TRIGLYCERIDES IN MAN INFECTED WITH SANDFLY FEVER

DAY	CONTROL (n=2)					INFECTED (n=3)				
	Cholesterol mg%				Triglyc.	Cholesterol mg%				Triglyc.
	Plasma	VLDL	LDL	HDL	mg/100 ml	Plasma	VLDL	LDL	HDL	mg/100 ml
- 7	159	18	130	-	134	158	16	130	-	84
- 3	134	6	99	30	83	161	20	110	31	81
0	136	9	101	27	124	155	13	114	28	79
1	152	28	94	30	120	154	16	111	28	62
2	149	22	106	22	128	151	5	113	29	60
3	132	8	100	24	110	131	17	91	24	59
4	152	13	112	26	126	122	12	94	16	62
5	142	15	103	25	110	133	23	92	18	72
6	142	4	112	27	94	136	14	97	22	82
7	142	10	111	23	100	145	13	100	22	81
8	153	17	110	25	85	140	17	95	29	82
10	156	17	110	29	71	149	16	109	24	89

a. VLDL = pre-β fraction.
LDL = β fraction.
HDL = α fraction.

b. R. S. Lees (Massachusetts Institute of Technology).

information concerning blood lipids and lipoprotein fractions. After stabilization during a 13-day control period on a constant liquid diet containing 40% fat, lipoprotein and blood lipid values were measured serially throughout the illness using methods previously described.[3] Quantitative lipid fractionation studies were also carried out by ultracentrifugation at M.I.T.[4]

Sandfly fever occurred in typical fashion after a 2-day incubation period. It was accompanied by anorexia. Percentage lipoprotein changes consisted of decreases in the pre-β fraction and a corresponding increase in the α fraction, which were most marked at the height of the febrile state. The first tendency to change, however, was noted prior to the febrile response or the drop in calories consumed. A significant decrease in serum cholesterol parralleled the decrease of the pre-β fraction. The α fraction tended to fall by days 7 and 8, and returned to preinfection levels during convalescense. No changes were seen in the β fraction.

In the ultracentrifugal examinations, the 3 fractions are indicated by their relative densities: high (HDL) = α, low (LDL) = β, and very low (VLDL) = pre-β. When these fractions were analyzed for their cholesterol content, it was found that the β fraction which transports cholesterol was decreased. Results are shown in Table I. Also, as may be seen here, triglyceride levels were also decreased in the infected men, beginning on day 1; they reached their lowest levels on day 3, returning to normal by days 6 and 7. Dr. Lees' findings agree with our observations of a marked decrease in serum cholesterol. Although no percentage changes were seen by us in the β fraction, this fraction was altered quantitatively, as shown by its decreased cholesterol content.

Summary, Part I:

In volunteers infected with sandfly fever there is a decrease in serum cholesterol and triglycerides which begins with the first 24 hr of infection and is more marked at the height of the febrile state. There is a decrease in the pre-β lipoprotein fraction and an increase in the α fraction at similar times. The latter begins to fall during the 7th and 8th days, returning to normal during convalescence. Ultracentrifugal studies show a decrease in the cholesterol content of the β fraction which is most marked on the 3rd and 4th days of illness.

Progress, Part II:

Since no infection-related studies were available to provide insight into the mechanisms of lipid kinetics, studies were begun on the interrelationship of lipid metabolism during infection using tracer compounds of a fatty acid in the experimental monkey model system. A single pulse dose of tritium-labeled palmitic acid was given to monkeys intoxicated with staphylococcus enterotoxin B (SEB) and Salmonella endotoxin. The labeled fatty acid was complexed to monkey albumin and injected 2 hr postchallenge. Blood samples were obtained at frequent intervals over a 2-hr period and analyzed for free fatty acid and triglyceride content. Control and experimental monkeys were examined concurrently and body temperatures were held constant within 1-2 degrees. Preliminary results show an increase in the disappearance rate of the labeled fatty acid and increased incorporation into triglycerides. These differences are

more marked in the endotoxin-intoxicated animals. These results will be subjected to computer analysis using a 2-compartmental model to define further the kinetics involved. The studies will also be expanded to include monkeys infected with pneumococcus and Salmonella organisms and the use of multi-compartmental computer analysis.

Summary, Part II:

Monkeys intoxicated with SEB and endotoxin show increased utilization of fatty acids and incorporation of labeled fatty acid into the triglyceride fraction in the liver.

Progress, Part III:

In an attempt to understand the nutritional aspect of host defense mechanisms and its relation to lipid metabolism during infection, studies were begun on Beagle dogs infected with infectious canine hepatitis (ICH) virus. A high caloric, high fat diet (the Military Stress Diet) is currently being used routinely in sentry dogs in Vietnam. Because of experimental work which has shown a depressed resistance with overnutrition and high fat diet,[5,6] dogs were divided into 2 groups. One group was fed regular Purina chow and the other the Military Stress Diet. There was a consistent weight gain in both groups, but a marked increase in subcutaneous fat in the high fat-fed dogs. After a stabilization period on each diet for 2 months, both groups were infected with ICH virus. Blood was drawn for determinations of serum lipids and lipoproteins.

In the high fat-fed animals, the disease course was much more progressive, severe, and had a more rapid mortality than in the control diet group. There was a marked increase in the pre-β (VLDL) fraction in the high fat-fed dogs prior to infection. Lipoprotein electrophoresis showed significant decreases in the pre-β fraction and corresponding increase in the α lipoprotein fraction during the illness. These changes were more marked in the high-fat diet group. Other lipid parameters were also studied; there was an increase in free fatty acids, and a decrease in triglycerides in both groups. Phospholipids were increased and total cholesterol was decreased in the control diet group. Light and electron microscopy studies were done on liver tissue from infected dogs. Preliminary results show no difference between them; typical lesions of ICH were seen.

Studies are also in progress characterizing the serum protein, glycoprotein and lipoprotein fractions in ponies and burros after immunization with attenuated Venezuelan equine encephalomyelitis virus (VEE). Burros showed a slight febrile response, accompanied by a decrease in percentage of serum albumin. Both species had increases in α_{1b} and α_2 globulins and a decrease in glycoalbumin and α lipoprotein.

Summary, Part III:

Beagle dogs fed a high-fat diet show decreased resistance to ICH infection. The reasons are unclear. Lipoprotein electrophoretic and serum lipid patterns

show changes compatible with an increased rate of lipolysis at the height of the illness in both groups. The Purina-fed dogs show increased phospholipids and decreased total cholesterol.

An examination of burros and ponies immunized with attenuated VEE vaccine show minimal changes in the serum protein, glycoprotein, and lipoprotein patterns.

Publications:

1. Beisel, W. R., and R. H. Fiser, Jr. 1970. Lipid metabolism during infectious illness. Amer. J. Clin. Nutr. (In press.)

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2. Klainer, A. S., W. R. Beisel, and W. K. Atkins. 1968. Determination of serum glycoproteins on cleared cellulose acetate strips. Amer. J. Clin. Path. 50:137-141.

3. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual Progress Report, FY 1969. p. 35 to 44. Fort Detrick, Maryland.

4. Frederickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins -- An integrated approach to mechanisms and disorders. New Engl. J. Med. 276:34-44.

5. Newberne, P. M. 1966. Overnutrition on resistance of dogs to distemper virus. Fed. Proc. 25:1701-1710.

6. Costello, R. L., L. W. Hedgecock, and T. R. Hamilton. 1962. Alteration of resistance of the rat to tuberculosis when maintained on an atherogenic diet. J. Exper. Med. 116:835-846.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 401:	Effect of Bacterial and Viral Infections on Host Cell Biosynthetic Mechanisms
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Michael C. Powanda, Captain, MSC, (I, II, III) Robert W. Wannemacher, Jr., Ph.D. (I, II,III) William S. Steinhart, Captain, MSC, (IV) Robert P. Nalewaik, Lieutenant, USNR, (V)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

64

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OL0809	2. DATE OF SUMMARY: 70 07 01		REPORT CONTROL SYMBOL: DD-R&E (AR) 636
3. DATE PREV SUMRY: 69 07 01	4. KIND OF SUMMARY: D. CHANGE	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8A. DISP'N INSTR'N: DE	8B. SPECIFIC DATA - CONTRACTOR ACCESS: [X] YES [ ] NO	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	401
b. [illegible]				
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Effect of bacterial and viral infections on host cell biosynthetic mechanisms

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 70	3	30
	CURRENT 71	3	30

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Steinhart, W. S. DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede each with Security Classification Code)
(U) Nucleotides; (U) Diurnal rhythm; (U) Pneumococcus; (U) Tryptophan; (U) Salmonella; (U) RNA; (U) Mycoplasma; (U) Chromatin template activity; (U) Sandfly fever; (U) Steroids

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To study alterations in nucleotide metabolism, RNA biosynthesis, and template activity of isolated chromatin in host cells during infection.

24 (U) Total nicotine-adenine dinucleotide (NAD) is measured in the tissues of mice subjected to a variety of conditions including infection with pneumococcus. The incorporation of radioactively labeled precursors of RNA into RNA isolated from various subcellular fractions is measured in the host during infection.

25 (U) 69 07 - 70 06 - Dietary tryptophan was manipulated in normal rats; hepatic NAD concentration reflected the availability of this amino acid. When pneumococcal infection was induced or animals were fasted, hepatic NAD levels decreased.

Kynurenine, one of the metabolites of tryptophan, was measured in volunteers infected with sandfly fever. Urinary excretion was increased, even though no additional tryptophan was given them and there was reduced food intake due to anorexia.

The rate of in vitro transcription of chromatin extracts was measured in mice. There is duirnal rhythm of chromatin template activity, and increase in it after infection with pneumococci, followed by a decrease.

A method that affords good separation of 16S and 22S RNA of Mycoplasma laidlawii has been developed. It shows promise for the study of RNA in other organisms and tissue culture lines.

Publications: Biophys. Soc. Abstracts 10:165a, 1970 (abstract).
Fed. Proc. 29:569, 1970 (abstract).

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 401: Effect of Bacterial and Viral Infections on Host Cell Biosynthetic Mechanisms

Description:

To study the mechanisms which control amino acid metabolism (with particular attention to that of tryptophan), protein synthesis, and the transcription of chromatin in normal and infected animals.

Progress, Part I:

Tryptophan and its metabolites have been demonstrated to affect polyribosome patterns and protein synthesis,[1,2] gluconeogenesis,[3] mitochondrial respiration[4] and lipid metabolism[5] in a variety of mammals. In addition, tryptophan is known to be a precursor of the pyridine nucleotides (NAD, NADH, NADP, NADPH), the prime redox entities of cellular metabolism, in man[6] as well as in other animals. Studies were therefore undertaken to elucidate the elements which control tryptophan metabolism. A recent report indicated that the conversion of tryptophan to CO_2 was regulated by substrate load *per se* rather than by alterations in tryptophan oxygenase activity.[7] The formation of NAD from tryptophan involves many of the reactions which are also a part of CO_2 production, specifically the metabolic sequence which begins with tryptophan oxygenase (TO) and is known as the kynurenine pathway.[8] Is it also primarily controlled by the availability of substrate? To answer this, 2 experimental approaches were employed.

In the first, young rats were fed synthetic amino acid diets containing 0, 1.5, 3.0 or 4.5 gm tryptophan/kg mix. The animals were kept on a schedule of 0600-1800 hours of light, and 1800-0600 hours of dark; they were sacrificed at either 0800 or 2000 hours on day 3 or day 10 to allow assessment of liver tryptophan and NAD concentration and TO activity. The tryptophan content of the livers on both days was essentially constant. TO activity was variable and in fact even showed an inverse relationship to dietary tryptophan at 0800 on day 10. Hepatic NAD concentration on the other hand always reflected the dietary level of tryptophan; Table I shows the correlation coefficients and their probabilities.

TABLE I

DAY	HOURS	CORRELATION COEFFICIENT r	PROBABILITY p
3	0800	0.52	< 0.01
3	2000	0.86	< 0.005
10	0800	0.79	< 0.005
10	2000	0.83	< 0.006

Correlation between hepatic NAD concentration and level of dietary tryptophan

The second approach made use of pulse-doses of tryptophan administered intraperitoneally (IP) to mice. Following a single 15-mg dose, liver tryptophan levels rose rapidly, within minutes, and so did assayable TO activity. Hepatic NAD concentration peaked at about 2 hr. The concentration of NAD and of tryptophan as well as the level of oxygenase activity in the liver were proportional to the amount of tryptophan injected.

Both approaches yielded data that strongly indicate that NAD synthesis from tryptophan is regulated by the availability of tryptophan rather than by fluctuations in TO activity. The pulse-dose studies however suggested that in the conversion of tryptophan to NAD, increases in TO activity did in fact occur. Whether these increases are necessary or coincidental cannot at the moment be determined.

Another conclusion to be drawn from the study is that rats as well as mice[9/] exhibit a diurnal variation in hepatic NAD concentration.

Summary, Part I:

Studies involving manipulation of dietary tryptophan in rats and pulse-doses of this amino acid in mice yield the same conclusion: hepatic NAD concentration reflects the availability of tryptophan in those animals which can use this metabolite as a precursor for pyridine nucleotides.

Progress, Part II:

Although increased tryptophan metabolism is noted in infections of bacterial, viral and rickettsial origin[10] and an increase in TO activity has been reported to occur in mice infected with pneumococci[11], there is no evidence of an increase in gross hepatic pyridine nucleotide concentration.

Rather, preliminary studies in rats and mice infected with 1-2 x 10^6 Diplococcus pneumoniae and then fasted from the moment of infection, indicate that a diminution occurs in hepatic NAD concentrations; most of the decrease can be attributed to decreased food intake. It should be noted that these measurements are merely an assessment of the quantity of NAD present and do not reflect the rates of synthesis or degradation of this compound.

Summary, Part II:

Hepatic NAD concentration falls in mice and rats following injection with 1-2 x 10^6 pneumococci. This decrease can be mimicked by fasting alone and thus does not seem to be due directly to infection.

Progress, Part III:

Prior experiments in volunteers demonstrated that bacterial, viral and rickettsial infections all increase the urinary output of some of the metabolites of tryptophan[10]. At that time an oral 3-gm dose of tryptophan was used to enhance the detectability of these substances.

A recent study with sandfly fever virus infection in man (Project FY 70-3) showed that it was possible to analyze for the metabolites of tryptophan in the urine without resorting to a prior pulse-dose of the amino acid and confirm that excretion of kynurenine is elevated during the febrile phase of infection. The increase in kynurenine excretion is observed when the volunteers have markedly reduced their food intake. This would suggest that the infection-related increase in metabolism of tryptophan is not related to the dietary level of this amino acid.

Further studies are planned in which the metabolites of tryptophan other than, and in addition to those of the kynurenine pathway will be measured in order to assess the effect of infection on pyridine nucleotide and serotonin metabolism.

Summary, Part III:

The urinary excretion of kynurenine was increased during sandfly fever infection in humans and was significant even though the volunteers had not received a tryptophan load and had reduced food intakes.

Progress, Part IV:

Chromatin has been isolated from livers of mice at various times for 23 hr following IP inoculation either with D. pneumococci or with saline. We have determined the rate of in vitro transcription of the chromatin extracts by incubating ^{14}C-labeled nucleotides with Escherichia coli DNA-dependent RNA polymerase and varying quantities of the chromatin, followed by determination of the ^{14}C incorporation into RNA. The polymerase was purified from frozen bacteria in this laboratory. Our results indicate (a) a diurnal rhythm of chromatin template activity in extracts from control animals, (b) an increase in template activity within 5 hr after infection, and (c) a subsequent decrease lasting through the remaining sampling period. The diurnal rhythmicity of normal hepatic chromatin template activity is therefore altered by generalized pneumococcal infection which may demand a different pattern of transcription (gene expression) in the liver.

Studies are in progress which utilize adrenalectomized mice to examine the possibility that steroid hormones have a role as mediators of the above effects. We also intend to examine RNA molecules synthesized in vivo and in vitro to try to detect and perhaps quantitate new RNA species whose synthesis is stimulated by infection.

Summary, Part IV:

Evidence has been obtained suggesting that early changes in protein synthesis in livers of infected mice may be due at least in part to effects of infection on transcription of genetic information.

Progress, Part V:

A method has been developed for separating the 16S and 22S components of RNA of Mycoplasma laidlawii. This method involves phenol extraction of the nucleic acids followed by their fractionation on a column of methylated bovine serum albumin adsorbed onto Kieselguhr. This method appears to be reproducible and might be useful for study of RNA from other organisms and from tissue culture lines.

Summary, Part V:

A method that affords good separation of the 16S and 22S components of RNA of M. laidlawii has been developed.

Presentations:

1. Steinhart, W. S. 1970. Alteration of rhythmic chromatin template activity in livers of pneumococcus infected mice. Presented at Fourteenth Annual Meeting of Biophysical Society, Baltimore, Md. 25-27 Feb 1970.

2. Powanda, M. C., and R. W. Wannemacher, Jr. 1970. Some aspects of tryptophan in the rat. Presented at Federation of American Societies for Experimental Biology, Atlantic City, N. J. 12-17 April 1970.

Publications:

1. Steinhart, W. S. 1970. Alteration of rhythmic chromatin template activity in livers of pneumococcus infected mice. Biophys. Soc. Abstracts 10: 165a. (Abstract).

2. Powanda, M. C., and R. W. Wannemacher, Jr. 1970. Some aspects of tryptophan in the rat. Fed. Proc. 29: 569 (Abstract).

3. Rapoport, M. I., W. R. Beisel, and R. B. Hornick. Tryptophan metabolism during infectious illness in man. J. Infect. Dis. (In press).

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5. Hirato, Y., T. Kawachi, and T. Sugimura. 1967. Fatty liver induced by injection of L-tryptophan. Biochim. Biophys. Acta 144:233-241.

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9. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual Progress Report, FY 1969. p. 59 to 66. Fort Detrick, Md.

10. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1968. Annual Progress Report, FY 1968. p. 21 to 22, Fort Detrick, Md.

11. Rapoport, M., G. Lust, and W. R. Beisel. 1968. Host enzyme induction of bacterial infection. Arch. Int. Med. 121:11-16.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 403:	Host-parasite Relationships in Arbovirus Infections
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Richard O. Spertzel, Major, VC Jerry D. Weil, Captain, VC J. Brent Rollins, Captain, VC Donald E. Kahn, Captain, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0812	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	403
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)
(U) Host-parasite relationships in arbovirus infections

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	5
FISCAL YEAR CURRENT 71	2	30

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede each with Security Classification Code)
(U) Encephalitis (VEE, EEE); (U) Virus diseases; (U) Arboviruses; (U) Yellow fever; (U) Teratology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate various interactions between animal hosts and arboviruses.

24 (U) Study transmissibility and susceptibility to arboviruses in a variety of animal species.

25 (U) 69 07 - 70 06 - No work was accomplished during this report period due to inability to work with infectious organisms in Building 1425. Work will resume during the coming year.

*Available to contractors upon originator's approval.

DD FORM 1498 1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 403: Host-parasite Relationships in Arbovirus Infections

Description:

Investigate various interactions between animal hosts and arboviruses.

Progress and Summary:

No work was accomplished during this report period due to inability to work with infectious organisms in the new medical laboratory. Work will resume during the coming year.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 01:	**Pathogenesis of Infection of Military Importance**
Work Unit No. 096 01 800:	**Biological Effects of Microbial Toxins**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Joseph C. Denniston, Jr., Captain, VC (I-V) Frank M. Calia, Major, MC (I) Donald E. Kahn, Captain, VC (III) Howard S. Kessler, Captain, VC (I, IV, V) Virginia G. McGann, Ph.D. (II, III) Richard O. Spertzel, Major, VC (I-V) James W. Stiles, Captain, MSC (II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

76

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OLO871	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	800
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Biological effects of microbial toxins

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	5	30
FISCAL YEAR CURRENT 71	5	30

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Kessler, H. S. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Staphylococcus; (U) Cardiotoxicity; (U) Endotoxin; (U) Hypersensitivity; (U) Shock

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To study the biological effects of microbial toxins.

24 (U) The effects of staphylococcal enterotoxin B (SEB) on animal hosts are measured by a number of parameters.

25 (U) 69 07 - 70 06 - Cardiotoxicity of SEB was evaluated in monkeys. Arrhythmia occurred only following high intravenous doses of crude product; purification apparently removed the impurity which produced it. Chronic infusion of SEB into monkeys was seen to affect pulmonary vascular endothelium; 6 of 9 monkeys that died had pulmonary congestion and edema.

An attempt was made to determine the mechanisms of SEB shock by direct visualization by light microscopy of the conjunctival blood vessels of monkeys. Preliminary results suggest the presence of hyperemia and capillary dilatation within 45 min.

Cutaneous hypersensitivity in SEB-experienced monkeys was demonstrable. Maximum sensitivity was too low for a practical test.

Publications: J. Appl. Physiol. 27:164-169, 1969.
Appl. Microbiol. 17:394-398, 1969.
Amer. J. Vet. Res. 31:507-514, 1970.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 800: Biological Effects of Microbial Toxins

Description:

Study the biological effects of microbial toxins in the animal host.

Progress, Part I:

In 1969, Vick et al[1] reported increased toxicity of staphylococcal enterotoxin B (SEB) product obtained from Lot 10-275 by the salting-out process. It appeared to be due to a marked primary cardiovascular effect in both the intact animal and in the detached, perfused heart. Although no incidence of direct cardiotoxicity was ever observed in any study dealing with the highly purified standard Lot 14-30 SEB, Vick's work raised the possibility that toxin prepared from Strain 275 organisms might be cardiotoxic. Such a possibility had never been eliminated. Thus, this study was designed to evaluate specifically the cardiotoxic effect of SEB.

Studies were conducted in monkeys, using several different lots of SEB. These were selected so as to represent SEB preparations with different degrees of purity, to include SEB processed by different methods, and to compare toxins derived from different strains of Staphylococcus. All monkeys were examined in advance to insure an absence of serological evidence of SEB immunity.

Any observed changes in electrocardiographic (EKG) pattern following the intravenous (IV) administration of SEB must be interpreted with the understanding that alterations are known to accompany changes in heart rate, and more importantly, that marked changes in these patterns occur as a consequence of lethal shock (in the absence of specific toxemia).

Extremely high doses (> 10 mg/kg, IV) of SEB produced from the various strains did not produce any EKG alterations within 15 min of challenge. This observation indicated that differences in the strain of Staphylococcus producing the enterotoxin could not be incriminated as the cause for the early EKG changes observed by Vick et al.[1] The terminal alterations that occurred were compatible with those changes observed in terminal shock of any etiology.

When crude (30-40% purity) SEB was studied, EKG changes developed within 15 min of toxin administration. These changes were seen only with extremely high doses of the crude preparations, and were seen with both heat-dried and vacuum-evaporated products. Typical findings included an initial tachycardia, aberrant conduction, atrioventricular (A-V) dissociation, third degree A-V block, bradycardia, ventricular fibrillation, and wandering atrial pacemaker.

Since these changes were observed very early, only at extremely high doses of impure products, and could not be reproduced with highly purified material obtained from the same strains, they can be ascribed, most logically, to extraneous staphylococcal products or impurities that had not been removed during partial purification procedures.

TABLE I. EFFECT OF SEB AND HEMORRHAGIC SHOCK ON THE EKG OF THE RHESUS MONKEY

NO. OF ANIMALS	TOXIN	DOSE mg/kg	NO. DEVELOPING CARDIAC ARRHYTHMIA 0-15 MIN POSTCHALLENGE
5	10-275 (52-68)	1	0
1	10-275 (52-68)	5	1
2	10-275 (52-68)	10	2
4	VE Blend 11-20(69)	10	4
2	44-275	15	0
2	14-30	10	0
2	Hemorrhagic shock	--	2

Summary, Part I:

The cardiotoxic effect of SEB has been evaluated. Cardiac arrhythmias developed only with high IV doses of crude SEB and appeared to be functions of extraneous staphylococcal products or impurities that were not removed during partial purification procedures.

Progress, Part II:

In 1966 Greisman et al[2/] reported that when bacterial endotoxins were infused IV at constant rates into healthy volunteers, the febrile and toxic responses returned to baseline within several hours, despite the continuing infusion. Such rapid refractoriness appears to represent a specific desensitization to endotoxin, since endotoxin-refractory rabbits exhibit unimpaired pyrogenic responsiveness to influenza virus, tuberculin and staphylococcal enterotoxin.

Studies were initiated to study the pathophysiology of enterotoxemia in the

rhesus monkey during long-term, continuous infusion of SEB. It is anticipated that this study will (a) lend evidence to the development of a refractory state with SEB, (b) identify target organs or tissue receptor sites for SEB, and (c) provide a model system for studying the course of enterotoxemia and a therapeutic approach to the disease.

Table II summarizes the dose rates, time of infusion and outcome of the animals in question. Chronic infusion of SEB at the rate of 0.01 or 0.02 μg/kg per min for 60- and 30-hr periods of administration, respectively, consistently resulted in a high incidence of mortality. Low-level chronic infusions of SEB exposed the test animals to a total dose of 15 μg/kg, which is only half the calculated median lethal dose (LD_{50}) of this toxin. Obviously, these animals did not develop a refractoriness to SEB as occurs with specific desensitization to endotoxin during constant IV infusion of the toxin.

TABLE II. CHRONIC INFUSION STUDIES

TOTAL DOSE (μg/kg)	DOSE/MIN (≃ μg/min)	SCHEDULED INFUSION TIME (hr)	DEATH/TOTAL
250	0.21	60	2/2
50	0.04	60	2/2
15	0.01	60	3/4
15	0.02	30	2/4
10	0.008	60	0/2
2	0.001	60	0/1

All chronically infused animals developed leukopenia about 2 hr following start of infusion. This was followed by a slight rise in white blood count (never reaching the control level) and then persistent leukopenia throughout the course of the infusion. This contrasts with the single-SEB-injection studies of Crawley et al[3] where the initial leukopenia was followed by persistent and rapid development of rebound leukocytosis. Crawley noted a marked increase in immature cells during the leukocytosis. The latter finding was not noted in this study.

There were no significant changes in serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), and lactate levels during low-level, chronic infusions of SEB. These findings differ somewhat from the findings of Crawley et al[4] in which an early rise in SGOT and BUN was seen. Additionally, we have noted an early drop in the packed cell volume which contrasts with the work of Rhoda et al.[5] Six of 9 animals that died during the chronic infusion studies had gross evi-

dence of pulmonary congestion and edema.

High-level infusions of fluorescein-tagged SEB (1 mg/kg/hr), coupled with electron microscopy, provide further evidence that a primary site of action of SEB is the pulmonary vascular endothelium. (See Work Unit No. 096 01 803).

Summary, Part II:

Chronic SEB infusion studies thus far are indicative of the basic action of SEB on vascular endothelium.

Progress, Part III:

Skin-testing for the presence of immediate hypersensitivity in rhesus monkeys to SEB was conducted in a selected group of animals. The study was designed to assess the feasibility of developing a quantitative bioassay system capable of detecting low concentrations of SEB. Animals were reinoculated at biweekly intervals to determine the sensitivity and reproducibility of the test. Much of the experiment was patterned after the work of Weiszer et al.[6]

Each animal was inoculated intradermally (ID) with 0.1-ml volumes of SEB in physiologic, pyrogen-free NaCl. The tests were run in the abdominal skin. A titration was performed by making a series of ID inoculations down the abdominal wall. Each test site contained 0.1 ml of NaCl containing 1/10 the toxin of the site immediately above it. The test was controlled with an ID inoculation of the NaCl diluent. Replicate tests were run simultaneously on each animal; one to the left and the other to the right of the midline.

The results of the testing program indicated that SEB-immunized rhesus monkeys displayed cutaneous hypersensitivity when inoculated ID with homologous antigen. Animals without previous experience to SEB were nonreactive to the skin test, but the initial testing program exposed each animal to > 22 μg SEB and rendered the animal sensitive to subsequent ID inoculations of toxin. The response pattern of selected monkeys is presented in Table III. Maximal sensitivity observed for the test was 0.001 μg SEB/0.1 ml. There was good reproducibility between replicate tests conducted simultaneously, and fair agreement in the sensitivity of the same animal on various test days. The development of skin-testing as a method for quantitation of SEB was discontinued when it was determined that the desired sensitivity of the bioassay could not be achieved.

TABLE III. SEROLOGIC STATUS AND CUTANEOUS SENSITIVITY OF RHESUS MONKEYS INOCULATED ID WITH SEB

TIME	TEST	RESPONSE BY MONKEY					
		K9-5	K9-6	J4-84	J4-112	J4-24	J4-103
Serology							
Oct 69	HI	-	-	320	320	640	1280
	Pptn	-	-	2	+	2	8
3 Dec 69	Pptn	-	-	1	2	2	8
16 Dec 69	Pptn	-	-	32	16	>32	>32
12 Jan 70	Pptn	-	-	16	32	>32	32
Cutaneous Hypersensitivity							
	μg/0.1 ml						
3 Dec 69	10	-	-	+	+	+	+
	1	-	-	+	+	+	+
	0.1	-	-	+	+	+	-
	0.01	-	-	-	±	-	-
	0.001	-	-	-	-	-	-
16 Dec 69	1	+	+	-	+	+	+
	0.1	-	-	-	+	+	-
	0.01	-	-	-	+	-	-
	0.001	-	-	-	-	-	-

Summary, Part III:

Cutaneous hypersensitivity in SEB-experienced rhesus monkeys could be demonstrated by ID inoculation of toxin. The maximal sensitivity achieved in the testing was 0.001 μg SEB/0.1 ml. It was determined that the bioassay of SEB in monkey skin would not be a practical method of detecting and quantitating low concentrations of the toxin.

Progress, Part IV:

The effect of certain pharmacological agents on the microcirculation in dogs has been studied by direct visualization of the capillaries of the mesenteric arterial system by some investigators.

Using a direct lighting microscope, we attempted direct visualization of the conjunctival vessels in the monkey to determine the mechanisms of shock of SEB. Time-lapse pictures were taken postinjection from 0 time to death.

Summary, Part IV:

An attempt was made to determine the mechanisms of SEB shock by direct visualization using light microscopy of the conjunctival blood vessels of the monkey. There is a suggestion that there is hyperemia and dilatation of the capillaries within the first 45 min of injection.

Progress, Part V:

A pharmacological approach to the study and treatment of enterotoxemia has been initiated in the primate. Initial studies have employed prechallenge prophylactic therapy with phenoxybenzamine, based on work by Vick[27] which demonstrated the efficacy of its use in endotoxin shock, and the beneficial effects of this drug in the treatment of pulmonary congestion reported by Sukhnandan.[87] It is believed that the effect upon pulmonary congestion is due, in part, to relaxation of the postcapillary pulmonary bed. This drug showed some beneficial effects when used during SEB toxemia in rabbits (see Work Unit No. 096 01 801).

Preliminary data in monkeys indicated that the degree of success utilizing phenoxybenzamine was closely related to dosage; dosages of > 0.75 mg/kg were ineffective in preventing death. However, doses between 0.5 mg/kg and 0.75 mg/kg have resulted in increased survival rates as compared to controls.

Phenoxybenzamine was not effective in preventing the development of pulmonary edema during the course of enterotoxemia in monkeys.

Preliminary work with isoproterenol has been unrewarding.

The study has been expanded to include continuous assessment of the animal's arterial blood pressure, central venous pressure, EKG, respiration rate, pH, PCO_2, PO_2, and blood lactate concentration. It is hoped that this study will markedly improve the chemotherapeutic approach.

Summary, Part V:

A pharmacological approach to therapy of SEB enterotoxemia in monkeys has been initiated.

Publications:

1. Elsberry, D. D., D. A. Rhoda, and W. R. Beisel. 1969. Hemodynamics of staphylococcal B enterotoxemia and other types of shock in monkeys. J. Appl. Physiol. 27:164-169.

2. Staab, E. V., J. Niederhuber, D. A. Rhoda, C. S. Faulkner, III, and W. R. Beisel. 1969. Role of the kidney in staphylococcal enterotoxemia. Appl. Microbiol. 17:394-398.

3. Rhoda, D. A., D. D. Elsberry, and W. R. Beisel. 1970. Fluid compartment alterations in the monkey with staphylococcic B enterotoxemia. Amer. J. Vet. Res. 31:507-514.

4. Rhoda, D. A. and W. R. Beisel. 1970. Lymph production during staphylococcic B enterotoxemia-induced shock in monkeys. Amer. J. Vet. Res. (In press.)

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7. Vick, J. A. 1964. Endotoxin shock in the primate: Treatment with phenoxybenzamine. J. Clin. Invest. 43:279-284.

8. Sukhnandan, R., and A. P. Thal. 1965. The effect of endotoxin and vasoactive agents on dibenzyline-pretreated lungs. Surgery. 58:185-196.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 801:	Mediators of Microbial Toxin Activity
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	David Auerbach, Captain, VC (I, II) Theordore S. Herman, Major, MC (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0872	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	801
b. [redacted]				
c. [redacted]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Mediators of microbial toxin activity

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 70	2	50
	CURRENT 71	2	50

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Herman, T. S. DA
NAME:

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Toxoid; (U) Plasminogen; (U) Metabolism; (U) Serum enzymes; (U) Phenoxybenzamine; (U) Toad bladder; (U) Water transport (U) Coagulation

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To evaluate the role of host mediators in the action of microbial toxins.

24 (U) Blood coagulation, metabolic, and hemodynamic parameters are studied in animal hosts challenged with staphylococcal enterotoxin B (SEB). Permeability of the toad bladder to osmotic movement of water is used to study the effects of SEB on transport processes.

25 (U) 69 07 - 70 06 - In the rabbit and monkey, SEB intoxication leads initially to a great increase followed by a decrease in plasminogen activator levels.

Phenoxybenzamine appears to prevent the metabolic alterations that accompany SEB intoxication in rabbits and to produce survival.

Serum from a monkey challenged with SEB potentiates vasopressin stimulation of osmotic water movement across the toad bladder.

*Available to contractors upon originator's approval.

DD FORM 1498 1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 801: Mediators of Microbial Toxin Activity

Description:

To evaluate the role of host mediators in the action of microbial toxins.

Progress, Part I:

Plasminogen activator levels as measured by the euglobulin lysis time test are markedly increased during the first 1 1/2 hr after intoxication with staphylococcal enterotoxin B (SEB) in the rabbit and monkey. Thereafter, the times are markedly prolonged and this condition persists through 24 hr. The latter changes may be the result of a depletion of plasminogen activator or plasminogen or an increase in blood antiplasmin levels. Current investigation centers on determining relative blood concentrations of these 3 factors. Plasmin is a highly active humoral agent which[1/] has been found to be a potent activator of the vasoactive kinins.

Summary, Part I:

Marked alterations in levels of a plasminogen activator occur during SEB intoxication.

Progress, Part II:

A pharmacotherapeutic approach to SEB toxemia has been initiated using chronically catheterized rabbits. The physiologic and metabolic parameters examined include: body temperature, central arterial (CAP) and venous pressures (CVP), arterial and venous PCO_2, PO_2, and pH, blood lactates and pyruvates, blood glucose and serum enzymes, serum glutamic oxalacetic transaminase (SGOT) and lactic dehydrogenase (LDH).

The physiological alterations indicated a progressively declining CAP and no change in the CVP. Blood lactates began to rise immediately postchallenge reaching peak values (5-6 x normal) by 9 hr. Blood pyruvates initially followed a parallel pattern but by 9-12 hr the levels began to fall with a resulting increase in the lactate: pyruvate ratios. Changes in blood gases were inconclusive at that time but a rapidly developing metabolic acidosis did occur in spite of a marked hyperventilation. Hyperglycemia developed initially but persisted only through 3 hr after which

hypoglycemia occurred. SGOT and LDH levels followed identical slopes with 10-fold plasma level increases seen by 9-12 hr, persisting until death. These metabolic changes along with the physiological alterations indicated that cellular damage was occurring early in the toxemia and that an increasing hypoxic state was being produced which culminated in the shock-like death of the animal.

To date, phenoxybenzamine prophylaxis has prevented death in 4 of 6 rabbits. In these 4, a severe hypotensive state was produced by the drug; following challenge with SEB a further fall occurred. However, in the 2 that died, the drug prevented appearance of the expected metabolic changes.

Summary, Part II:

Prophylactic phenoxybenzamine protected 4 of 6 rabbits from death due to SEB intoxication; these animals failed to show the typical metabolic changes seen in the animals that succumbed.

Progress, Part III:

The isolated urinary bladder of the toad (Bufo marinus) is being used as a model system to study the effect of SEB on the transepithelial transport processes.

SEB at concentrations of 0.025- 100 µg/ml had no effect on baseline or vasopressin-stimulated osmotic water flow. However, serum from a monkey challenged intravenously 30 min earlier with 300 µg/kg SEB caused an enhancement of vasopressin-stimulated water flow when compared to serum from the same monkey obtained prior to SEB challenge. Experiments are underway to identify the serum factor responsible for this phenomenon and to determine if there is a possible mediator of the toxic effects of SEB.

Preliminary studies have been started to study the effect of SEB on oxygen utilization in isolated toad bladder mucosal cells.

Summary, Part III:

Serum from a monkey challenged 30 min earlier with SEB has been found to potentiate vasopressin-stimulated osmotic water movement.

Publications:

None

LITERATURE CITED

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 01:	Pathogenesis of Infection of Military Importance
Work Unit No. 096 01 802:	In Vivo Distribution of Microbial Toxins
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Pathology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	James B. Moe, Captain, VC (I-III) Ulysses McElyea, Jr., Captain, VC (I-III) Hubert J. Wolfe, Lt Colonel, MC (IV) Thelton W. McCorcle, Captain, VC (IV)
Reports Control Symbol:	RCS-MEDDH-288 (R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0873	70 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	802
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) In vivo distribution of microbial toxins

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	40
FISCAL YEAR CURRENT 71	2	40

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Enterotoxin; (U) Staphylococcus; (U) Immunofluorescence; (U) Immunoenzymatic techniques; (U) Isotopic tracers; (U) Antigen; (U) Antibody; (U) Horseradish peroxidase

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the transport and localization of microbial toxins in animal tissues and the pathogenesis of the corresponding intoxications following various routes of challenge.
24 (U) Enzyme tagging techniques for protein labeling are developed. Enzyme, fluorescein, and radioisotope tagged and untagged toxins are administered to susceptible species by various routes. Toxin distribution is determined by appropriate methods. Distributions noted are compared with similar studies using horseradish peroxidase.
25 (U) 69 07 - 70 06 - Radial immunodiffusion assay of circulating staphylococcal enterotoxin B (SEB) levels after intravenous challenge correlates with previous results of other assay methods. A late elevation of circulating SEB near death was found. Microscopic changes were studied in animals dying from intoxication. Lesions most often associated were peribronchial and perivascular edema of the lung, occasional swollen Kupffer cells and dilated spaces of Disse in the liver.

Vascular permeability induced in the nonsusceptible rat by high doses of SEB is probably due to trace quantities of alpha hemolysin. In comparing rate and transport route of various proteins from pulmonary parenchyma of monkeys, the innocuous proteins, horseradish peroxidase and bovine serum albumin, were removed slowly via the right lymphatic duct, not by transport across the alveolar capillary wall. SEB appears to be only initially removed from the lung by the right lymphatic duct. After 3-4 hr exposure due apparently to an increase in permeability, transport across the alveolar capillary occurred. While the site of action remains unknown, it is suggested that this represents a toxic effect of SEB and may play a major role in the pathogenesis of SEB toxemia

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Pathogenesis of Infection of Military Importance

Work Unit No. 096 01 802: In Vivo Distribution of Microbial Toxins

Description:

Study of clearance of SEB in monkeys and localization of SEB in animal tissues and the pathogenesis of the intoxications.

Progress, Part I:

Clearance of staphylococcal enterotoxin B (SEB) from the circulation of monkeys (Macaca mulatta) after intravenous (IV) challenge was studied utilizing the radial immunodiffusion technique.

Intact nonexperienced (SEB-negative) monkeys were given 500 μg SEB/kg body weight IV and were bled serially from 30 min postinjection. At 30 min postinjection the circulating level was 2.0-4.5 μg SEB/ml serum; by 1-2 hr it had decreased to < 0.5 μg/ml serum. Three of 7 monkeys exhibited an apparent rise of circulating SEB near death.

Thirty minutes after challenge with 500 μg/kg of SEB, an intact experienced (SEB-positive) monkey had 34 μg SEB/ml serum; and this level dropped to 1.4 μg SEB/ml serum by 1 hr remaining so until death at 2½ hr.

Two nephrectomized SEB-negative monkeys had approximately 7 μ g SEB/ml serum at 30 min after IV challenge with 500 μg/kg. By 3 hr this level was reduced to a negligible quantity. Both monkeys exhibited a terminal elevation of about 0.5 μg/ml 1 hr prior to death from SEB intoxication.

One nonexperienced nephrectomized monkey given Thorotrast (ThO_2), followed by IV challenge with 500 μg SEB/kg demonstrated essentially the same clearance curve as nonexperienced nephrectomized monkeys challenged with SEB without prior Thorotrast blockade.

Two nephrectomized SEB monkeys were given 1000 μg fluorescein-labeled SEB/kg body weight IV and bled at 15-min intervals until sacrifice at 1 hr. One was given Thorotrast 1 hr prior to SEB challenge. Both monkeys had similar clearance curves.

Summary, Part I:

Determination of circulating levels of SEB by the radial immunodiffusion assay technique in monkeys under various experimental conditions indicates that SEB is cleared rapidly from the circulation of intact nonexperienced animals and that nephrectomy and circulating anti-SEB antibody delays clearance. Thorotrast blockade does not alter the clearance rate. These results correlate with previous work utilizing other assay techniques. There is occasionally a rise in the level of circulating SEB near the time of death from SEB intoxication.

Progress, Part II:

Nephrectomized monkeys, with and without Thorotrast blockade, were given 1000 µg fluorescein-labeled SEB/kg body weight and sacrificed at 1 hr post-injection. Sections of brain, liver, lung, lymph nodes, spleen and other organs were processed through paraffin, sectioned at 5 µ, cleared, and examined with the ultraviolet microscope. Fluorescent activity attributable to fluorescein-labeled SEB was found in Kupffer cells of the liver and reticuloendothelial cells of the choroid plexus of the brain. Fluorescence could not be demonstrated in the visceral and peripheral lymph nodes, spleen or lung. Quantitative differences between the fluorescent activity of the Thorotrast blockaded and nonblockaded animals could not be demonstrated.

Summary, Part II:

When studied in nephrectomized monkeys, fluorescence attributable to fluorescein-labeled SEB was found in the reticuloendothelial cells of the choroid plexus of the brain and in liver Kupffer cells but not in reticuloendothelial cells of other organs. Thorotrast blockade did not appear to alter uptake of fluorescein-labeled SEB.

Progress, Part III:

Necropsies were done on monkeys dying from SEB intoxication and those euthanized during the course of SEB intoxication (see also Work Unit 096 01 800). Gross examination of the tissues revealed only parasitic infestations of the lung mite (*Pneumonyssus simicola*) and the nematode *Esophagostomum spp*. Occasional monkeys had grossly recognizable pulmonary edema. Microscopically there was a variable amount of perivascular and peribronchial pulmonary edema. Pulmonary edema was occasionally absent and at times it was severe. Kupffer cells of the liver were occasionally swollen and the spaces of Disse were slightly dilated. Other lesions seen, such as pulmonary thromboemboli, were attributable to indwelling venous catheters. Animals given Thorotrast had phagocytized pigment in reticuloendothelial cells of the spleen, liver, and lymph nodes.

Summary, Part III:

Lesions observed in monkeys intoxicated with SEB were similar to those

previously described. Perivascular and peribronchial pulmonary edema were most commonly observed. Kupffer cells were swollen and the spaces of Disse were slightly and inconstantly dilated.

Progress, Part IV:

A study of possible vascular effects of SEB in the nonsusceptible adult rat (200-300 gm) was performed using the cremasteric muscle as a model, and carbon black as the vascular tracer. Varying quantities of SEB (10-1000 μg), lot 14-30, were injected intrascrotally in 0.1-ml volume on one side, and an equal volume of normal saline or histamine diphosphate (1 mg/ml) as negative or positive control on the other side. The animal was sacrificed at 1 hr. The cremasteric muscle was processed for light microscopy[1] and evaluated for an increase in permeability at any point in the vascular tree.

For the studies in a susceptible species anesthetized, 2.5-4 kg M. mulatta were used. The lung was the model organ; the vascular tags were radio-labeled SEB (^{125}I-SEB) and bovine serum albumin ^{125}I-BSA[2] or horseradish peroxidase (HRP). All monkeys used for SEB challenges were free of SEB hemagglutinating (HA) antibodies. Surgical cannulation of the femoral vein, thoracic duct, and right lymphatic ducts were performed;[3] the tracer employed was introduced via tracheostomy into the tracheobronchial tree through a catheter in 1-ml volumes. Doses administered in this fashion were 100-300 μg/kg of labeled-SEB or -BSA and 1-5 mg of HRP. Lymph was obtained by gravity flow 10-in below the heart. Over an 8-hr period, lymph and blood samples were collected at 30-min intervals and radioactive determinations for labeled SEB and BSA were made as previously described.[4] Standard biochemical assays of peroxidase activity were performed. Eight hours after intratracheal instillation, the animal was sacrificed and selected tissues, including kidney, lung, and adjoining lymph nodes were removed and processed for light microscopic localization of the vascular tracer, employing emulsion autoradiography or histochemistry (HRP).

In the rat, vascular leakage induced by SEB occurred only at extremely high concentrations (500-1000 μg/0.1 ml). This activity was unaffected by antihistamines, but was partially inactived by heat. In contrast to the histamine reaction, which affects the postcapillary venule, high SEB dosages produced increased permeability in the entire capillary bed, resembling the response noted with purified staphylococcal α-hemolysin (prepared, purified, and assayed by COL Metzger, Pathology Division) where as little as 0.01 unit of α-hemolysin produced increased capillary permeability. It seemed possible that the vascular leakage associated with high doses of SEB in the rat could be due to traces of residual α-hemolysin in the purified SEB.

SEB was handled differently by the monkey following intratracheal instillation than were the two innocuous proteins (BSA and HRP). For SEB, activity was detected in lymph from the right duct within the first 30 min and rose rapidly to peak at 3-4 hr postinstillation. In contrast, during this

interval no activity was detected in the thoracic duct lymph. At 3½-4 hr, there was a precipitous and sudden evolution of activity in the blood. The activity in blood rapidly rose to parallel levels in the right duct lymph.

For BSA and HRP the evolution of low activity occurred in the right duct lymph at 6-7 hr. Blood and thoracic duct lymph remained negative. Autoradiography demonstrated the localization of ^{125}I-SEB in the cytoplasm of pulmonary alveolar macrophages, histiocytes or peripheral sinuses, and medulla of regional lymph nodes and convoluted renal tubules. BSA and HRP were detected in pulmonary alveolar macrophages and histiocytes of the lymph nodes but not in renal tubular epithelium.

Summary, Part IV:

The vascular permeability induced in the nonsusceptible rat by high doses of SEB is probably due to trace quantities of α-hemolysin. In comparing the rate and avenue of transport of various proteins from the pulmonary parenchyma of M. mulatta, it would appear that with the innocuous proteins, HRP and BSA, slow removal occurs via the right lymphatic duct, not by transport across the alveolar capillary wall. In contrast, SEB appears only initially to be removed exclusively from the lung by the right lymphatic duct. After 3-4 hr exposure, due apparently to an increase in permeability of the alveolar capillary structure, transport across the alveolar capillary wall occurred. While the site of action in the alveolar wall remains unknown, it is suggested that this represents a toxic effect of SEB and may play a major role in the pathogenesis of SEB toxemia.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 01:	**Vulnerability of Man to Biological Agents**
Work Unit No. 096 01 803:	**Subcellular Biological Effects of Microbial Toxins**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Bacteriology and Pathology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Peter G. Canonico, Captain, MSC (I) Matthew Van Zwieten, Captain, VC (I) James W. Stiles, Captain, MSC (II-VII)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0896	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 05 19	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	01	803
b. CONTRIBUTING	[redacted]			
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Subcellular biological effects of microbial toxins

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	30
FISCAL YEAR CURRENT 71	2	30

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21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede each with Security Classification Code)

(U) Enterotoxin; (U) Staphylococcus; (U) Lysosomes; (U) Enzymes; (U) Pulmonary endothelium

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the subcellular effects and distribution of microbial toxins and other informational molecules.

24 (U) A variety of techniques, e.g. fractionation, electron microscopy are used to study subcellular action of toxins.

25 (U) 69 07 - 70 06 - Fluorescein-labelled toxin appears to be incorporated into kidney cell lysosomes. The intracellular distribution of the toxin closely resembles that of beta-glucuronidase and cathepsin D.

Following intravenous administration of staphylococcal enterotoxin B (SEB), rabbit lung shows an increased activity of 2 lysosomal enzymes, beta-glucuronidase, and acid phosphatase between 2 and 8 hr after exposure. Isolated mitochondria from rabbit lung, kidney and liver demonstrate a marked inhibition of cytochrome oxidase activity after in vitro addition of SEB. Preliminary histochemical studies have also shown an inhibition of cytochrome oxidase activity, which is more pronounced in the lung, than in liver or kidney.

SEB intoxication by intravenous infusion results in a vesicularization of the endothelial cells lining many pulmonary alveolar capillaries. The release of hydrolytic enzymes from disrupted localized leukocytes may cause the breakdown and, so, the pulmonary edema. This mechanism may apply to other bacterial toxins that cause a pulmonary necrosis. Neutralization of leukocyte hydrolyses is suggested as therapy.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 01: Vulnerability of Man to Biological Agents

Work Unit No. 096 01 803: Subcellular Biological Effects of Microbial Toxins

Description:

Study the subcellular effects and distribution of microbial toxins and other informational molecules.

Progress, Part I:

The lysosome was found by de Duve to be part of a class of subcellular organelles that function as an intracellular digestive system.[1] These organelles have also been shown to be responsible for the inactivation and destruction of toxins and microorganisms.[2]

In certain instances, the interactions of toxins and biologically active molecules with lysosomes have been shown to precede morphological and functional cellular changes. Such alterations are thought to result from an increased permeability or complete disruption of lysosomal membranes; this permits hydrolytic enzymes to be released into the cytoplasm indicating autocatalytic reactions.

Partial lysosomal digestion of disease producing microorganisms has been demonstrated to be a mechanism by which infective material is released into the cytoplasm of susceptible cells.[3] Furthermore, incomplete digestion of toxic molecules and infectious microorganisms may lead to congestive enlargement of lysosomes with impairment of other intracellular digestive functions.[4]

In the study of disease mechanisms the prevailing concept is that each intoxication or successful proliferation of an invading microorganism is the result of some failure of the defensive functions of lysosomes. It is obvious, therefore, that research on the function of lysosomes is of primary importance in understanding the interaction of cells with toxins and infective microorganisms.[2]

Projects initiated during the latter part of this fiscal year were designed to determine the effects and distribution of microbial toxins and other biologically active molecules on lysosomes and other subcellular organelles.

The subcellular distribution of toxins as well as other biologically active compounds are being investigated by fluorospectrophotometric

determinations of fluorescein-labelled compounds, and by fractionation of tissue homogenates by differential, ratal and zonal centrifugation, as described by Bowers and de Duve[5] and Canonico and Bird.[6]

The effects of toxins and biologically active compounds on the activity of such intracellular marker enzymes as ATPase, β-glucuronidase, acid phosphatase, cytochrome oxidase, urate oxidase, glutamic oxaloacetic transaminase, lactic acid dehydrogenase, catalase, cathepsin D, and RNase, are being investigated by biochemical, histochemical and electron microscopic techniques.

Preliminary tissue fractionation experiments indicate that fluoresceinated staphylococcal enterotoxin B (SEB) administered intravenously (IV) to normal rabbits becomes incorporated within the lysosomes of kidney cells. This intracellular distribution of SEB closely resembles the distribution of the lysosomal enzymes β-glucuronidase and cathepsin D. However, the possible association of a portion of the toxin with mitochondrial membranes cannot be ruled out on the basis of the present data.

As shown in Table I, rabbit lung, but not kidney, shows a significant increase in the specific activity of β-glucuronidase and acid phosphatase

TABLE I. LYSOSOMAL ENZYME ACTIVITY IN SEB-TREATED RABBITS

		UNITS/mg PROTEIN/MIN ± SE	
TISSUE	TIME (Hr)	Acid phosphatase	β-glucuronidase
Lung	Control	1.48 ± 0.20	25.7 ± 2.4
	2	2.24 ± 0.54	46.4 ± 5.9
	8	3.31 ± 0.15	43.2 ± 6.3
	16	1.40 ± 0.04	34.2 ± 1.6
Kidney	Control	1.44 ± 0.14	10.2 ± 1.5
	2	1.69 ± 0.42	11.0 ± 0.9
	8	2.50 ± 0.10	11.7 ± 1.2
	16	1.26 ± 0.09	11.4 ± 0.6

between 2 and 8 hr after intoxication with an IV dose of SEB. Cytochrome oxidase activity of isolated mitochondria from rabbit lung, kidney and liver was found to be inhibited by in vitro addition of SEB. Maximum inhibition of 70 - 80% was obtained by incubation of mitochondria with SEB for 10 min at 37 C. The inhibition of cytochrome oxidase by SEB, like that produced by protamine and poly-L-lysine, may be a function of the cationic properties of the molecule which competes with reduced cytochrome c for binding sites on the enzyme. In vivo experiments, however, have failed to demonstrate any alterations in the activity of liver, kidney and lung cytochrome oxidase, suggesting that the inner structure of whole mitochondria is not accessible to SEB. On the other hand, preliminary histochemical studies in lung tissue slices of rabbits injected with SEB have indicated a reduction of cytochrome oxidase activity, which suggests that the toxin may, in fact, have access to the inner structures of intact mitochondria. Experiments are now underway to elucidate the significance of these observations.

Using the techniques of Trouet,[7] a biologically nondegradable detergent (Triton WR-1339) has been used to isolate rabbit liver lysosomes by flotation in discontinuous sucrose gradients. Hydrolases extracted from these lysosomes have been shown to hydrolyze SEB. Sephadex column chromatography is currently being employed in an attempt to characterize the products of SEB hydrolysis.

Summary, Part I:

Experiments have been initiated and preliminary data obtained to determine the intracellular distribution of SEB and its effects on the activity of marker enzymes of subcellular organelles.

Progress, Part II:

Examination of monkey intestine for evidence of mitochondrial lesions. Swollen mitochondria having disrupted cristae membranes were shown by Merrill and Sprinz[8] to be characteristic of monkey jejunum that had been intoxicated per os with staphylococcal enterotoxin B (SEB). These observations seemed significant in light of the mitochondrial mechanism as reviewed by Kadis, et al,[9] of the murine toxin of Pasteurella pestis. Accordingly, experiments were undertaken to both verify the in vivo intestinal mitochondrial lesion and to further test the effect in vitro of SEB on mitochondria isolated from different tissues obtained from SEB susceptible monkeys and rabbits. Unpublished data from Sugiyama (reported in Crawley, et al[10]) did, however, indicate that the oxidative phosphorylation of mitochondria was not inhibited by SEB.

Monkeys were given 1 mg of SEB through a gastric tube. After emesis had occurred, the animals were euthanized and intestinal segments were prepared for electron microscopy, thick sections being cut from all segments. Upon examination with the light microscope, no evidence was seen of the

vacuolated cytoplasm of the intestinal epithelial cells as was observed by Merrill and Sprinz.[8] Electron microscopic examination of thin sections cut from selected segments resulted in the finding of only normal mitochondria.

Summary, Part II:

SEB did not affect intestinal mitochondria in monkeys.

Progress, Part III:

The effect of SEB on mitochondrial electron transport. Mitochondria were isolated from monkey and rabbit intestine, liver and kidney by homogenization of tissue and differential centrifugation of the homogenates through 0.25 M sucrose solutions containing Mg++ and Tris-Cl. Oxygen uptake was measured with a "Clark" type oxygen electrode. Both succinate and reduced diphosphopyridine nucleotide were used as substrates. It was found that while standard inhibitors such as cyanide inhibited the respiration, no concentration of SEB did so whether added in the reaction mixture before or during measurement. In addition, minced fresh whole intestine did not show significant respiratory inhibition.

Summary, Part III:

SEB did not inhibit electron transport in monkey and rabbit mitochondria of intestinal, hepatic and kidney tissues.

Progress, Part IV:

The effect of SEB on ligated intestinal loops. To determine whether an intestinal lesion caused by SEB could cause the diarrhea seen in SEB intoxication, the cholera toxin assay of Burrows and Musteikis[11] was used. In this assay system rabbit ileum is tied off in loops in the body cavity. Toxin is instilled into the ligated segments before closure of the abdominal wall and the animal is subsequently euthanized with fluid accumulation indicating the amount of cholera toxin introduced.

SEB was injected into rabbit intestinal loops in 10-100 μg amounts. No fluid accumulation was observed in the loops in each of 2 animals. These results suggested that the diarrhea seen in enterotoxin toxemia was not a direct effect of SEB on the intestinal wall and that the toxin could be affecting the digestive tract via one or more intermediate steps.

Summary, Part IV:

SEB did not cause fluid accumulation in ligated rabbit intestinal loops *in vivo*.

Progress, Part V:

Evidence for long-term maintenance of low levels of SEB in serum. If SEB was not exerting a direct effect on intestine, it was possible that a

blood circulating intermediate substance was. Together with Captain Denniston of Animal Assessment Division, transfusion experiments were performed where serum samples from SEB intoxicated animals were given to recipient animals. As it was known that SEB is rapidly removed from the circulation,[12] it is thought that serum taken after about the first hr would have no SEB and could contain only an intermediate toxic compound. However, it was found that all the transfusion recipients -- even 1 receiving blood after 55 hr -- developed hemagglutination-inhibiting (HA) titers to SEB. Essentially, this biological assay demonstrated that very low levels of SEB remained in the bloodstream over the course of the toxemia. These amounts were smaller than could be detected with reliability by serological or radiochemical means.

Summary, Part V:

Very low levels of SEB could be detected in monkey serum samples up to 55 hr following intoxication.

Progress, Part VI:

Response of monkeys of sustained blood levels of SEB. The long-term presence of SEB in the circulation of monkeys led us to propose that this presence had a role in the course of the toxemia. Consequently, long-term IV SEB infusions were undertaken. It was hoped that such a route of administration would decrease the role of the kidney as seen in toxin removal from the circulation and also increase the incidence and severity of physiological and morphological lesions.

It was found (see Work Unit 01 800) that a sustained leukopenia occurred that exactly corresponded to the increase and steady state level of the SEB circulating. In addition, morphologic changes were noted in the lung.

Summary, Part VI:

Monkeys exhibited consistent clinical courses of SEB toxemia when infused over long periods with very low toxin amounts.

Progress, Part VII:

Endothelial breakdown of pulmonary capillaries. Finegold[13] reported pulmonary interstitial edema, endothelial emboli, and endothalial cell changes to be associated with SEB single dose intoxication. We have found that our control tissues are characterized by most of the lesions reported by him to have resulted from SEB intoxication. The exceptions have been the perivascular edema, and the slight vesicularization of the capillary endothelium.

We have observed more extensive effects through infusing similar doses (30 μg/kg) over 30-hr periods. The endothelial cells of the pulmonary capillaries were extensively separated into vesicles but this only occurred

in some capillaries. Disrupted leukocytes were seen to be closely associated with these broken endothelial cells. The leukocyte cell contents were also observed leaking into the lumen of a capillary having a disrupted wall. This led us to propose that the hydrolytic enzymes of disrupted leukocytes are responsible for the breakdown of the endothelial cells resulting in pulmonary edema. A speculative mechanism explains these phenomena:

1. SEB reacts preferentially with leukocytes causing their removal from the circulation.

2. The leukocytes stick in some vascular beds and the lung is one of these.

3. The leukocyte plasma membrane ruptures releasing hydrolytic enzymes and other cell contents.

4. The cell contents disrupt structures such as pulmonary endothelial cells directly and may affect other areas indirectly.

Since pulmonary endothelial damage is important in many bacterial infections, such a mechanism, if proven, could have general applicability. For instance, protection against pulmonary damage could be effected by immunologically, or chemically, blocking the leukocytic enzymes responsible for the damage possible in many bacterial infections. Work is continuing to extend this promising line of investigation.

Summary, Part VII:

SEB intoxication by IV infusion results in a vesicularization of the endothelial cells lining many pulmonary alveolar capillaries. The release of hydrolytic enzymes from disrupted localized leukocytes may cause the breakdown of endothelial cells and thereby lead to pulmonary edema. This mechanism may apply to other bacterial toxins that cause a pulmonary necrosis. Neutralization of leukocyte hydrolyses is suggested as therapy.

Publications:

None

LITERATURE CITED

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2. de Duve, C., and R. Wattiaux. 1966. Functions of lysosomes. Ann. Rev. Physiol. 28:435-492.

3. David-Ferreira, J. F., and R. A. Manaker. 1965. An electron microscope study of the development of a mouse hepatitis virus in tissue culture cells. J. Cell Biol. 24:57-78.

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13. Finegold, M. J. 1967. Interstitial pulmonary edema. An electron microscopic study of the pathology of staphylococcal enterotoxemia in rhesus monkeys. Lab. Invest. 16:912-924.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 002:	Evaluation of Efficacy of Experimental Vaccines in Man
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Medical, Virology, and Microbiology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Peter J. Bartelloni, Lt Colonel, MC (I-III, V) Robert W. McKinney, Lt Colonel, MSC (I, IV) Francis E. Cole, Jr., Ph.D. (I, IV) Helen H. Ramsburg, B.S. (I-IV) Nemesio M. Francisco, Major, MC (III) John D. Marshall, Jr., Colonel, MSC (V)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0L0829	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	002
b. Contributing				
c. Contributing	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Evaluation of experimental vaccines in man

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	190
FISCAL YEAR CURRENT 71	2	190

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vaccines; (U) Immunization; (U) Encephalitis, equine (VEE, EEE, WEE); (U) Globulin; (U) Q fever; (U) Adenovirus

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Evaluate experimental vaccines developed by various contractors, organizations or other governmental agencies.

24 (U) Test vaccines are given to experimental animals, and when considered safe, to volunteers.

25 (U) 69 07 - 70 06 - Inactivated Western equine encephalitis (WEE) vaccine was evaluated in man by 3 dosage schedules. Serologic responses were adequate for 1 year. Use of the vaccine as a booster in 43 persons with prior WEE experience demonstrated its high immunogenicity.

Clinical and serological responses were evaluated in volunteers given an inactivated Rift Valley fever vaccine. No significant clinical reactions or meaningful changes in clinical laboratory values were observed. Significant mean SNI developed by day 42, and persisted through day 90. The vaccine was shown to elicit a satisfactory response when used as a booster.

Chikungunya vaccine, inactivated, tissue culture origin Lot E-20 was evaluated in volunteers. It was found to be safe and of low reactogenicity. Antigenicity will be evaluated following completion of serological tests.

Results of in vivo neutralization tests on sera obtained from "at-risk" personnel who had received the attenuated VEE vaccine (TC 83/2-9) 5 to 6 years before, documented the persistence of antibody in these individuals.

Publication: Amer. J. Trop. Med. 19:123-126, 1970.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 002: Evaluation of Efficacy of Experimental Vaccines in Man

Description:

Evaluation of experimental vaccines developed by various organizations, contractors, or other governmental agencies.

Progress, Part I:

Evaluation of Inactivated Western Equine Encephalitis Vaccine: Clinical and serological responses to Western equine encephalitis (WEE) vaccine, inactivated, tissue culture origin, Lot 1-1967 were evaluated in volunteers in two 12-month studies (Project No. FY 69-3, FY 69-4).[1/] Serological results have been completed.

Table I shows serum neutralization indices (SNI) for the entire year. The 2 different dosage schedules for FY 69-3 and the single schedule for FY 69-4 are indicated. The majority of subjects failed to achieve significant SNI 14 days after the first dose of vaccine. The mean SNI 28 days after the first dose of vaccine was 1.7 for Group 1 and 1.8 for Group II. Fourteen days after the second dose of vaccine (day 42) the mean titer was 2.4 for Group I and 2.6 for Group II, with all subjects exhibiting significant SNI. There was no significant difference in the range of neutralizing antibody titers between the 2 groups. The mean SNI and ranges for both groups remained at these acceptable levels through day 360 with the exception of one individual in Group I whose titer dropped below 1.7 after day 42 and persisted at the lower level throughout the period of the study.

The majority of subjects from FY 69-4 failed to achieve significant SNI by 28 days after the first dose of vaccine. However, by day 42 the mean SNI was 2.5 and all subjects had achieved significant titers. The mean SNI remained at a significant level thereafter through day 360. The SNI of sera of one volunteer were < 1.7 on days 56, 90 and 180.

Thus all 3 dosage schedules appear to produce a similar immunogenic response. There is a suggestion of a slight delay in reaching significant SNI levels in the 6 men given 2 doses of 0.25 ml each.

TABLE I. NEUTRALIZATION INDICES OF VOLUNTEERS WITHOUT PRIOR WEE EXPERIENCE TO 2 DOSES OF INACTIVATED WEE VACCINE (LOT 1-1967) GIVEN 28 DAYS APART

DAY[a]	LOG_{10} SNI (RANGE)		
	Group I (0.5ml+0.5ml) (FY 69-3) n=8	Group II (0.5ml+0.25ml) (FY 69-3) n=7	FY 69-4 (0.25ml+0.25ml) n=6
0 (V)[a]	0	0	0
14	1.3 (0.6-2.0)	1.4 (1.2-1.6)	1.4 (0.8-2.3)
28 (V)	1.7 (1.2-2.1)	1.8 (1.4-2.4)	1.4 (1.1-2.1)
42	2.4 (1.8-2.9)	2.6 (1.8-3.1)	2.5 (1.7-2.9)
56	2.4 (1.4-3.4)	2.5 (2.1-3.1)	2.3 (1.5-3.2)
90	2.2 (1.4-3.3)	2.5 (2.1-3.0)	2.2 (1.3-2.8)
180	2.1 (1.5-2.5)	2.4 (2.0-2.9	2.2 (1.2-3.1)
270	2.2 (1.6-2.9)	2.2 (1.8-2.9)	2.7 (1.9-3.1)
360	2.1 (1.5-2.8)	2.4 (1.9-3.0)	2.6 (1.8-3.0)

a. Vaccine administered.

Immunization of "At-Risk" Personnel: Following completion of the preceding portion of the study, the vaccine was administered to 21 laboratory workers with no known prior WEE experience and who were at-risk to laboratory infection. Two dosage schedules were employed and evaluated as part of a long-term program concerned with the establishment of a rational, rather than an empirical, immunization scheme. Twelve subjects were given vaccine in doses of 0.5 ml each 28 days apart, while the remaining 9 were inoculated with 0.5 ml of vaccine on day 0, but only 0.25 ml of vaccine of day 28. Our data (Table II) clearly indicate that there is no significant difference between the serological responses of persons in Group I (92% $\geq$ 1.7 logs) and those in Group II (88% $\geq$ 1.7 logs) 28 days after the second dose of vaccine. Forty-three[2] additional workers, who had previously received an earlier WEE vaccine,[2] were given a 0.1 ml booster dose intradermally of the present vaccine. Problems of availability of these "at-risk" workers permitted only a serological evaluation of their response.

Of the 43 "at-risk" workers used for evaluation of the efficacy of the 0.1-ml booster dose of vaccine (Table III), the majority had received a basic series of 3 doses plus several annual booster doses of an earlier WEE vaccine;

TABLE II. SNI OF 21 AT-RISK PERSONNEL WITHOUT WEE EXPERIENCE, ADMINISTERED 2 DOSES OF WEE VACCINE 28 DAYS APART

GROUP (DOSAGE SCHEDULE) ml	LOG_{10}SNI	CONTROL	NUMBER RESPONDING 28 DAYS AFTER VACCINE DOSES 1	2
I (0.5 + 0.5)	3.0-4.0		0	4
	2.0-2.9		7	6
	1.7-1.9		0	1
	1.0-1.6		5	1
	<1.0	12	0	
II (0.5 + 0.25)	3.0-3.9			2
	2.0-2.9		3	6
	1.7-1.9		2	0
	1.0-1.6		3	1
	<1.0	9	1	0

TABLE III. SNI IN 43 WEE-EXPERIENCED, AT-RISK PERSONNEL ADMINISTERED 0.1 ML BOOSTER DOSE OF WEE VACCINE

LOG_{10} SNI	RESPONSE			
	Before booster dose		28 days after booster dose	
	%	No.	%	No.
≥ 4.0	0	0	2	1
3.0-3.9	0	0	23	10
2.0-2.9	9	4	61	26
1.7-1.9	2	1	9	4
1.0-1.6	21	9	5	2
< 1.0	68	29	0	0

none had been vaccinated within 6 months before receiving our vaccine. Approximately 88% of these persons had SNI < 1.7 logs at the time of booster inoculation whereas only about 5% remained at this level 28 days after the booster vaccination. This represents a serologic conversion of 95% of those with low titers, which in light of the data presented on the nonexperienced volunteers and "at-risk" workers documents well the immunogenicity of the new WEE vaccine.

Summary, Part I:

Formalin-inactivated WEE vaccine of chick embryo cell culture origin was evaluated in 21 volunteers, 21 "at-risk" laboratory workers (nonexperienced to WEE) and 43 WEE-experienced laboratory investigators. The majority of nonexperienced individuals converted serologically (significant level of $\geq$ 1.7 logs) and remained so for 1 year. Dosage schedules of 0.5 + 0.5 ml and 0.5 + 0.25 ml elicited essentially the same responses. A group of 6 volunteers given 0.25 + 0.25 ml also converted serologically, but with a slight delay in the attainment of higher titer. However, by the end of 1 year the mean SNI and range of this group was comparable to the other 2 groups.

Progress, Part II:

Evaluation of Inactivated Rift Valley Fever Vaccine: Clinical and serological responses to Rift Valley Fever (RVF) Vaccine, Inactivated, Tissue Culture Origin, NDBR 103-6 (National Drug Co.) were evaluated in volunteers. The initial study was an acceptability trial in which 3 non-Whitecoat, nonexperienced volunteers participated (Project No. FY 69-9). Each individual was administered 2 doses 28 days apart of 0.5 ml each of RVF vaccine in the deltoid region of the arm. Subjects were observed 24 and 48 hr after each inoculation of vaccine for local and systemic reactions. Only 1 of the 3 subjects developed discomfort at the site of injection, malaise and headache approximately 8 hr following administration of the first dose of vaccine, which persisted for about 5 hr. Serological evaluation was not made.

Clinical and serological responses to RVF vaccine were then evaluated in 16 volunteers (Project No. FY 69-10). The immunization schedule was the same as for FY 69-9. Four subjects served as controls receiving a placebo of 0.5 ml of Isotonic Sodium Chloride for Injection, Sterile, U.S.P. Subjects were housed on a closed metabolic ward; they were observed at least twice daily for local and systemic reactions for a period of 10 days after the initial dose of vaccine. After the second dose they were observed at 24 and 48 hr. Each subject had daily white blood cell, differential, hematocrit and platelet counts, as well as urinalysis and electrocardiograms (EKG). Frequent serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), direct and indirect serum bilirubin, blood urea nitrogen (BUN) and alkaline phosphatase determinations were obtained during the inpatient portion of the study. Blood was drawn to obtain serum for neutralization tests prior to immunization and on days 28, 42, 56, 90, 180, 270, and 360.

SNI were determined by the "constant serum-virus varying" method. Swiss mice, Webster strain from the Fort Detrick Animal Farm were inoculated via the intraperitoneal route with equal mixtures of pre- or postvaccination serum and 10-fold dilutions of Entebbe strain RVF virus. Animals were observed for deaths for 10 days. Fifty percent endpoints were determined by the method of Reed and Muench.[2/]

No significant local or systemic reactions developed upon administration of 2 doses of RVF vaccine. No meaningful changes in clinical laboratory values occurred during the period of study, and no significant EKG abnormalities were observed.

Serum neutralization tests have been completed through day 90 with sera obtained from vaccinees; results are shown in Table IV. The majority of volunteers did not attain significant levels of neutralizing antibody until day 42, or 14 days after the second dose of vaccine. The mean SNI on days 42, 56 and 90 were comparable. By day 42, 11 of 15 subjects (73%) had attained significant levels and by day 56, 12 of 15 (80%) had achieved significant neutralizing antibody. However, by day 90 only 9 of 16 individuals (56%) had SNI $\geq$ 1.7 logs.

Immunization of "At-Risk" Personnel: Forty-nine RVF-experienced, "at-risk" subjects were given a 0.5-ml booster dose of RVF vaccine 5-8 years after completion of a basic series and a booster dose of a similar vaccine.[3/] Eight individuals (16%) had one or more systemic complaints which consisted predominantly of headache (10%), malaise (6%), feverishness (6%), and generalized pruritus (4%); symptoms subsided in 48 hr. One individual complained of soreness at the injection site which subsided by 48 hr. No local signs were observed at the site of injection, no febrile reactions occurred. Sera were obtained from 45 of the 49 persons for SNI determinations 19-21 days following the booster. As indicated in Table V, 49% of these individuals had SNI < 1.7 logs prior to the administration of the booster dose. Nineteen to 21 days following the booster dose of vaccine only 16% had SNI < 1.7 logs.

TABLE IV. NEUTRALIZING ANTIBODY RESPONSES[a] IN 16 VOLUNTEERS WITHOUT PRIOR RVF EXPERIENCE TO THE ADMINISTRATION OF 2 DOSES OF 0.5 ML EACH OF RVF VACCINE 28 DAYS APART

VOLUNTEER	LOG_{10} SNI BY DAY AFTER THE FIRST DOSE				
	14	28	42	56	90
SBA	1.9	1.9	3.0	2.8	2.5
SLB	0.7	0.6	2.4	2.4	2.6
REE	2.6	2.1	ND[b]	2.0	2.5
VVE	0.4	0.9	1.7	1.0	1.0
RBH	1.0	0.9	0.5	0.9	0.4
KWJ	0	2.3	1.4	1.4	1.9
FLL	2.0	1.4	2.5	2.5	2.1
MVM	1.1	1.0	2.0	2.0	1.5
JAR	2.2	2.4	2.0	2.2	1.6
RWR	1.5	1.3	2.3	2.2	2.0
EJR	2.1	1.4	2.1	2.3	1.6
GLS	1.4	1.9	2.5	2.0	2.0
JLS	0.8	1.2	1.5	2.3	1.2
DRS	1.7	1.8	2.4	2.5	2.5
DES	0.9	1.4	1.5	1.7	2.4
CRR	1.2	1.9	2.1	ND	1.1
No. $\geq$ 1.7 logs	6	7	11	12	9
Mean	1.3	1.5	2.0	2.0	1.9
Range	(0-2.6)	(0.6-2.4)	(0.5-3.0)	(1.0-2.8)	(0.4-2.6)

a. Control day levels were < 1.0.
b. ND = not done.

TABLE V. NEUTRALIZING ANTIBODY TITERS IN 45 RVF-EXPERIENCED, "AT-RISK" INDIVIDUALS ADMINISTERED 0.5 ML BOOSTER DOSE OF RVF VACCINE

LOG_{10} SNI	RESPONSES			
	Before Booster Dose		19-21 Days Postbooster Dose	
	%	No.	%	No.
≥4.0	18	8	51	23
3.0-3.9	11	5	16	7
2.0-2.9	13	6	13	6
1.7-1.9	9	4	4	2
1.0-1.6	9	4	9	4
<1.0	40	18	7	3

Summary, Part II:

Clinical and serological responses were evaluated in 16 volunteers administered 2 doses of 0.5 ml each of RVF vaccine 28 days apart. Neither significant clinical reactions, nor meaningful changes in clinical laboratory values were observed.

Significant mean SNI developed by day 42 and persisted through day 90. Seventy-three and 80% of subjects developed significant titers by day 42 and 56 respectively; although by day 90 only 56% had significant SNI. Antigenicity of the vaccine was further demonstrated in 49 "at-risk" experienced subjects given a booster dose.

Progress, Part III:

Evaluation of Inactivated Chikungunya Vaccine: Clinical and serological responses to Chikungunya vaccine, formalin-inactivated, tissue culture origin, dried, Lot E-20 (Walter Reed Army Institute of Research, Washington, D. C.) were evaluated in volunteers. The initial study (Project No. FY 70-4) was an acceptability trial to determine and characterize the extent of local and systemic reactions. Six non-Whitecoat volunteers were divided into 3 groups of 2 each; 2 received 0.1 ml of vaccine intradermally, 2 received

0.5 ml of vaccine subcutaneously, and 2, 1.0 ml of vaccine subcutaneously. Each subject was examined for local and systemic reactions at 24 and 48 hr following the administration of vaccine; none developed. There was no serological evaluation.

In the evaluation study (Project No. FY 70-5) 16 volunteers were divided into 2 groups of 8 each, designated Groups I and II. Volunteers in Group I received 2 doses of 0.5 ml each of Chikungunya vaccine 28 days apart. Vaccine was inoculated subcutaneously in the deltoid region of the arm. Two additional volunteers served as controls and received 0.5 and 1.0 ml, as appropriate, of Isotonic Sodium Chloride for Injection, Sterile, U.S.P., in a similar manner. All subjects had daily white blood cell, differential, hematocrit, and quantitative platelet counts, as well as daily urinalysis and EKG performed under standard conditions. Frequent SGOT and SGPT, direct and indirect bilirubin, BUN and alkaline phosphatase determinations were made. All patients were observed at least twice daily for local and systemic reactions following the first dose of vaccine. After the second dose of vaccine volunteers were observed at 24 and 48 hr on an outpatient basis. Blood was drawn to obtain serum for neutralization and hemagglutination inhibiting tests prior to immunization and on days 14, 28, 42, and 56; additional blood will be obtained on days 90, 180, 270, and 360.

Local, febrile and systemic reactions were not observed in either group after the administration of 2 doses of vaccine. Clinical laboratory values remained within normal limits, and no significant changes in EKG were recorded.

Results of serological tests are not yet available.

Summary, Part III:

Chikungunya vaccine, inactivated, dried, Lot E-20, was evaluated in volunteers. The vaccine was found to be of low reactogenicity when administered in 2 doses of 0.5 ml or 1.0 ml 28 days apart. No changes in clinical laboratory determinations occurred. Serological data are not yet available.

Progress, Part IV:

Persistence of Neutralizing Antibody to Venezuelan Equine Encephalomyelitis Virus in Vaccinated Personnel: Sera were obtained from 89 "at-risk" workers who had received the attenuated Venezuelan equine encephalomyelitis (VEE) vaccine (TC 83/2-9)[4,5] 5-6 years before. SNI were determined by the "constant serum-varying virus" method. One to 3-day old mice were inoculated intracerebrally with equal mixtures of pre- or post vaccination ($\geq$ 5 year) sera and $\log_{10}$ dilutions of Trinidad strain VEE virus. Fifty per cent endpoints were determined 5 days postinoculation using the method of Reed and Muench.[2]

As indicated in Table VI, 33% of the individuals had significant levels of neutralizing antibody (i.e. SNI $\geq$ 1.7) at the time of immunization. However, some of these subjects had received repeated doses of an inactivated VEE vaccine, while others had incurred laboratory infections. Not apparent from the table is the fact that 84% of persons (49 of 59) with a low or negative initial SNI (i.e. $\leq$ 1.0 logs) had a rise in antibody to a significant level which persisted throughout the study period. Most significantly, 5 to 6 years following their vaccination, 84% (75 of 89) of these workers still had significant SNI; generally those with low titers were poor or marginal responders to initial vaccination. These data, in concert with a lack of any VEE laboratory infections subsequent to employment of this vaccine, document well the efficacy of the attenuated VEE vaccine with respect to its capacity to induce long-lasting, solid protection.

TABLE VI. SERUM NEUTRALIZATION INDICES OF 89 "AT-RISK" PERSONNEL AT $\geq$ 5 YEARS POSTVACCINATION WITH ATTENUATED VEE VACCINE

LOG_{10} SNI	RESPONSES			
	Day 0 Prevaccination		> 5 Years Postvaccination	
	%	No.	%	No.
3.0-$\geq$4.0	4	4	23	20
2.9-2.9	19	17	49	44
1.7-1.9	10	9	12	11
1.0-1.6	24	21	14	12
<1.0	43	38	2	2

Summary, Part IV:

Neutralization tests on sera obtained from "at-risk" personnel who had received the attenuated VEE vaccine 5-6 years previously documented the persistence of antibody in these persons.

Progress and Summary, Part V:

Thirty volunteers were administered 1.0 ml of Plague Vaccine in May 1970; booster doses will be administered at 3 and 6 months. The purpose of this study is to determine clinical reactions and to obtain long-term serological data.

Publication:

Bartelloni, P. J., R. W. McKinney, T. P. Duffy, and F. E. Cole, Jr. 1970. An inactivated Eastern equine encephalomeylitis vaccine propagated in chick embryo cell culture. II. Clinical and serologic responses in man. Amer. J. Trop. Med. 19:123-126.

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1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual progress report, FY 1969. p. 87 to 102. Fort Detrick, Maryland.

2. Reed. L. J., and H. Muench. 1938. A simple method for estimating fifty percent endpoints. Amer. J. Hyg. 27:492-493.

3. Randall, R., C. J. Gibbs, C. G. Aulisio, L. N. Binn, and V. R. Harrison. 1962. The development of a formalin-killed Rift Valley fever virus vaccine for use in man. J. Immun. 89:660-671.

4. Berge, T. O., I. S. Banks, and W. D. Tigertt, 1961. Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea-pig heart cells. Amer. J. Hyg. 73:209-218.

5. McKinney, R. W., T. O. Berge, W. D. Sawyer, W. D. Tigertt, and D. Crozier. 1963. Use of an attenuated strain of Venezuelan equine encephalomyelitis virus for immunization in man. Amer. J. Trop. Med. 12:597-603.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 003:	Chemoprophylaxis and Therapy of Infectious Diseases of Potential Biological Warfare Significance
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Medical
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Peter J. Bartelloni, Lt Colonel, MC Nemesio M. Francisco, Major, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0830	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	003
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Chemoprophylaxis and therapy of infectious diseases of potential biological warfare significance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 09	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: NA d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	1	10
	CURRENT 71	1	20

19. RESPONSIBLE DOD ORGANIZATION

NAME:* USA Medical Research Institute of Infectious Diseases

ADDRESS:* Fort Detrick, Md 21701

RESPONSIBLE INDIVIDUAL

NAME: Crozier, D.

TELEPHONE: 301 663-4111 Ext 5233

20. PERFORMING ORGANIZATION

NAME:* Medical Division
USA Medical Research Institute of Infectious Diseases

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME:* Bartelloni, P. J.

TELEPHONE: 301 663-4111 Ext 6135

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Francisco, N. M.

NAME: DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Prophylaxis; (U) Therapy; (U) Infectious diseases; (U) Antibiotic

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Assess the effect of microbials and various drug regimens in various infectious diseases.

24 (U) Various drugs are tested in volunteers under strict protocol conditions.

25 (U) 69 07 - 70 06 - During the year, no tests were performed. This is a work unit needed for future work as required.

Publication: Amer. J. Med. Sci. 258:203-208, 1969.

*Available to contractors upon originator's approval.

DD FORM 1498 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 003: Chemoprophylaxis and Therapy of Infectious Diseases of Potential Biological Warfare Significance

Description:

Assess the effect of antimicrobials and various drug regimens in various diseases.

Progress and Summary:

During the year, no tests were performed.

Publication:

1. Bartelloni, P. J., F. M. Calia, H. L. Ley, Jr., B. H. Minchew, and W. R. Beisel. 1969. Absorption and excretion of two chloramphenicol products in humans after oral administration. Amer. J. Med. Sci. 258:203-208.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 02:	**Prevention and Treatment of Biological Warfare Casualties**
Work Unit No. 096 02 004:	**Studies in Combined Antigens**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Bacteriology and Animal Assessment
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	William A. Christmas, Captain, MC (I) Harry G. Dangerfield, Lt Colonel, MC Richard O. Spertzel, Major, VC (II) Frank E. Chapple, Captain, VC (II)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OL0831	2. DATE OF SUMMARY* 70 07 01	REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA-CONTRACTOR ACCESS [X] YES [ ] NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	004
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Studies in combined antigens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
63 04	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	90
FISCAL YEAR CURRENT 71	2	80

19. RESPONSIBLE DOD ORGANIZATION
NAME:* USA Medical Research Institute of Infectious Diseases
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RESPONSIBLE INDIVIDUAL
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Christmas, W. A.
TELEPHONE: 301 663-4111 Ext 7341
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Dangerfield, H. G.
NAME: Spertzel, R. O. DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede each with Security Classification Code)
(U) Encephalitis, equine (VEE, EEE, WEE); (U) Rift Valley fever; (U) Chikungunya; (U) Q fever; (U) Antigens; (U) Immunization; (U) Vaccines

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Determine the feasibility of combining various immunizing antigens and establish the compatability, optimal dose, best schedule for administration, and efficacy of the combinations.

24 (U) Various antigens will be mixed in a variety of proportions and given to experimental animals for challenge studies. Promising combinations will be tested further and eventually may be tested in volunteers.

25 (U) 69 07 - 70 06 - An experimental pentavalent vaccine containing inactivated WEE, EEE, Rift Valley fever, Chikungunya, and Q fever has been prepared. The combination was found to be physically compatible but hypertonic. The mixture met the established U. S. Public Health Service criteria for safety. Bioassays of the monovalent vaccines and infectious organisms are in progress to provide quantitative reference values for the pentavalent vaccine.

Completion of a combined vaccine study of VEE and yellow fever has been held in abeyance pending occupation of the Phase I facility.

Publications: J. Immun 102:1220-1227, 1228-1234, 1969.
Clin. Res. 18:45, 426, 1970 (abstracts).

*Available to contractors upon originator's approval.

DD FORM 1498 1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare

Work Unit No. 096 02 004: Studies in Combined Antigens

Description:

Determine the feasibility of combining various immunizing antigens and establish the compatability, optimal dose, best schedule for administration, and efficacy of the combinations.

Progress, Part I:

Currently, vaccines developed for defense against biological weapons are routinely administered to at-risk personnel in dosages and schedules independent for each vaccine. Combination of these antigens into a single multivalent vaccine would afford obvious advantages.

Criteria to be met by such a product include the following: (1) the vaccine should be contained in one vial, (2) the vaccine should be administered in a minimum number of injections, (3) all components in the combination should provide protection equivalent to that of each monovalent vaccine included, and (4) safety and acceptability should meet the standards established by the U. S. Public Health Service.

For initial studies we elected to employ only inactivated particulate vaccines. These were Western Equine Encephalitis (WEE), Eastern Equine Encephalitis (EEE), Rift Valley Fever (RVF), Chikungunya (CHIK) and Q Fever (Q). Each of these monovalent preparations has been approved by the Army Investigational Drug Review Board for use in man.

It was arbitrarily decided that the antigenic mass of each component in the mixture would be the same as that of the corresponding monovalent vaccines currently being given to at-risk personnel. Since the administration of a combination containing 1 human dose of each component (as usually reconstituted) would require an injection of 4.0 ml, reduction in volume was desirable. Consequently, the 4 lyophilized vaccines were reconstituted with fluid Chikungunya vaccine so that 1 ml of the final mixture contained approximately 1 human dose of each component.

A consideration of primary importance was physical compatibility of the combination. The appearance of the combined vaccine was similar to reconstituted monovalent preparations, and there was no visible precipitate or change in turbidity. The experimental product had a pH of 7.65 and specific gravity of 1.023; Na and K concentrations were 408 and 16 mEq/L respectively. It is obvious that a final product would require adjustment

for tonicity. The newly reconstituted pentavalent vaccine was tested in mice and guinea pigs and was found to be safe according to PHS criteria.

A single animal model was desirable in order to eliminate species variability, but no experimental animal was susceptible to all 5 organisms. For this reason Swiss mice were selected for assays with CHIK, and the golden (Syrian) hamster for WEE, EEE, RVF and Q.

Baseline data for the individual vaccines were required as reference values for the pentavalent product. Therefore, a median effective dose (ED_{50}) for each vaccine and a median lethal dose (LD_{50}) or median fever dose (FD_{50}) for each organism were necessary. These studies are currently in progress.

Summary, Part I:

An experimental pentavalent vaccine containing inactivated WEE, EEE, RVF, CHIK, and Q has been prepared. The combination was found to be physically compatible but hypertonic. The mixture met the established PHS criteria for safety. Bioassays of the monovalent vaccines and infectious organisms are in progress to provide quantitative reference values for the pentavalent vaccine.

Progress and Summary, Part II:

The completion of a combined vaccine study, in which the serologic responses of monkeys are examined to determine if 2 live viral vaccines, Venezuelan equine encephalomyelitis and yellow fever, interact antagonistically or synergistically when administered at various times relative to each other, has been held in abeyance, pending operational use of the new laboratory.

Presentations:

1. Irvin, W. S., and C. P. Craig. Virus alteration of presumptive tests of delayed hypersensitivity. Presented at Southern Section, American Federation for Clinical Research, New Orleans, La. 29-31 January 1970.

2. Irvin, W. S., and C. P. Craig. Prevention of *in vitro* expression of delayed hypersensitivity (DH) by virus. Presented at American Federation for Clinical Research, Atlantic City, N. J. 2-3 May 1970.

Publications:

1. Craig, C. P., S. L. Reynolds, J. W. Airhart, and E. V. Staab. 1969. Alterations in immune responses by attenuated Venezuelan equine encephalitis vaccine. I. Adjuvant effect of VEE injection in guinea pigs. J. Immun. 102:1220-1227.

2. Airhart, J. W., G. S. Trevino, and C. P. Craig. 1969. Alterations in immune responses by attenuated Venezuelan equine encephalitis vaccine. II. Pathology and soluble antigen localization in guinea pigs. J. Immun. 102:1228-1234.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 005:	Studies on Antibody Production and Their Binding Properties
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Mary H. Wilkie, M.S.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0886	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	005
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Studies on antibody production and their binding properties

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: NA d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	11
FISCAL YEAR CURRENT 71	1	11

19. RESPONSIBLE DOD ORGANIZATION

NAME:* USA Medical Research Institute of Infectious Diseases

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: DA

NAME:

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Antigens; (U) Antibody; (U) Binding strength; (U) Serology

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study antigen binding properties of antibodies as a means of assessment of protective efficacy of experimental vaccines and therapeutic antisera against bacterial and viral agents.

24 (U) Establish an arbitrary scale to express binding affinities in place of presently used equilibrium constants, utilizing different biological or physical chemical techniques. Results will be related to protection tests.

25 (U) 69 07 - 70 06 - Rabbits were immunized with bovine serum albumin (BSA) with or without Freund's adjuvant and the sequential antisera obtained were analyzed for IgM and IgG antibodies by gel filtration chromatography and I-131 labelled BSA binding capacity. Early IgM production was followed by an IgG response which was sustained in those animals immunized with BSA and adjuvant. A secondary stimulation 6 months later produced an anamnestic IgG response followed by a new primary IgM/IgG sequence. As reported, use of adjuvant in the primary inoculation leaves less memory for the secondary response.

Degradation of BSA by pronase was inhibited by IgG antibody, but not by IgM. Inhibition of antigen hydrolysis by IgG was related to binding capacity of the antibody and appeared to be competitive in type.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 005: Studies on Antibody Production and Their Binding Properties

Description:

Study kinetics of the immune response and antigen binding properties of antibodies as a means of assessment of protective efficacy of experimental vaccines or therapeutic antisera against bacterial and viral agents.

Progress:

Employing bovine serum albumin (BSA) as a classical soluble protein antigen, evaluation of the kinetics of the immune response of rabbits by quantitative binding methods are continuing.[1/]

A new series of rabbits were immunized with 2 inoculations, each containing 25 mg BSA with or without Freund's adjuvant. Sequential bleedings were analyzed for IgM and IgG antibodies by fractionation with Sephadex G-200 gel filtration. Antibody levels were determined employing the binding of ^{131}I-labelled antigen by the Farr technique;[2/] in addition, passive hemagglutination (HA) of BSA coupled rabbit red cells[3/] by the same antisera fractions was evaluated by means of antigen binding values.

The primary response (induced by the first injection of BSA) proceeded through an identical sequence of events in all rabbits until about the 14th day. IgM was produced between days 5 and 7; IgG, though present in trace amounts during this period, increased significantly between days 7 and 14. Therefore, animals immunized with BSA and Freund's adjuvant differed from those immunized with BSA alone. Without adjuvant, antibody synthesis appeared to cease abruptly at about 14 days; the antibody level decreased thereafter in parallel with the normal catabolic rate of normal γ-globulin in the rabbit. With adjuvant, IgG synthesis was sustained at a higher level for some months, suggesting that BSA was recycled through the antibody synthesis system.

During the primary immunologic response, an erratic quantitation of antibody levels by HA titration was clearly evident. In the same antiserum, submicrogram quantities of IgM, determined by binding, could yield HA titers of $\geq$1:64, while a milligram of early IgG might yield a titer of 1:8. As the immune response matured, HA titers of IgG increased although absolute quantities remained the same as determined by binding and precipitins. In other words, HA efficacy increased with maturation of the immune response

without an increase in absolute quantity of IgG. Since HA titers were unrelated to quantities of either antibody class, they were completely unreliable as a quantitative procedure.

After 6 months, the rabbits which had been immunized with BSA alone had no detectable antibody; they were restimulated with 10 mg BSA to test the amount of residual "memory." Sequential bleedings beginning on day 4 were tested in the manner described above. IgG synthesis was apparent on that day, became maximal on day 8, and was 25% lower on day 14. In contrast to the primary immunization sequence, IgM appeared as the second antibody on day 14, followed by a second rise of IgG on day 19. It appeared that the IgG "anamnestic" response on days 4-7 combined with the booster to produce a delayed new response by day 14, simulating the sequence observed in the primary response.

The "memory" testing of sera of rabbits immunized with BSA and Freund's adjuvant is not complete. After 6 months, these animals were still producing some 7S antibody of IgG and IgA class. As described in the previous report,[17] preliminary testing indicates that immunization with adjuvant leaves less "memory" than the above series. It also indicates that stimulation with 10 mg of BSA is in excess and produces a mixture of "anamnestic" IgG and a simultaneous primary response. Thus, in both groups, the secondary, or anamnestic, response is also a highly kinetic series of events.

In these studies, with the use of a foreign, rapidly degraded protein, BSA, whose only demonstrable biological effect is antigenicity, a baseline of the normal immune response in the rabbit has been established. The amounts of antigen processed and stage of development of the immune response in this species can now be predicted by analysis of a few samples of antisera. In the rabbit, the pattern of an immune response to other antigens can be assessed and the effects of variables evaluated, e.g., toxicity, by comparison to the established baseline. Evaluation of the excellent data of Fukazawa, et al[57] is an example of the application of this principle. These authors immunized rabbits with Salmonella typhimurium cells or with lipopolysaccharide or lipopolysaccharide-protein complex extracted from these organisms. The IgM and IgG pattern of antibody response was determined by agglutination, but was not quantitated. By comparison with the normal baseline, the data clearly indicated that: (1) rabbits responded to the O antigens in a normal time frame for each step of the immune sequence; (2) the O antigen complexes of this organism were relatively easily degraded, although less so than BSA; (3) the rabbit was capable of responding to the protein complex with a complete immune response through the IgG stage, and (4) neither the bacterial vaccine nor the lipopolysaccharide presented a sufficient quantity of antigen to complete this response; with these vaccines, the immune response remained at the IgM stage which is characteristic of immunization with submicrogram quantities.

The toxicity of gram negative O antigens limits the amounts of vaccine which can be employed. The above evaluation suggests that addition of enough detoxified polysaccharide-protein complex to these vaccines might enhance their effectiveness with safety. With the use of a biologically inert antigen, such a baseline can be structured for any species under study. By comparison to a normal baseline, the response to unknown antigens or vaccines can be evaluated more comprehensively and efficiently.

Unique properties of antibodies may explain mechanisms of immune clearance of antigens and produce better in vitro methods of evaluating the immune response. The observation that antibody protected up to 90% of a protein antigen (BSA) in preformed complexes or in whole antiserum from enzyme attack was presented in an earlier report.[1/] BSA, rabbit anti-BSA sera, and the enzyme, pronase, were used in these studies.

It was demonstrated that resistance of BSA to pronase hydrolysis was conferred by IgG antibody, but not by IgM. Moreover, IgM appeared to inhibit the protection of IgG when tested in combination.

At constant levels of total protein concentration, serum, BSA and pronase, it was found that enzyme resistance of BSA was directly proportional to the absolute quantity of antibody, as measured by ^{131}I-BSA binding. It seems, for any antigen binding value, that fixed percentages of complex, or specific antigen:antibody (Ag:Ab) molar ratios, are resistant to enzyme.

As reported by McKernan and Ricketts,[4/] interaction of BSA with soluble DEAE-dextran yields Ag/Ab-like complexes which simulate the classical precipitin curve. In our studies, when such complexes were treated with pronase, BSA was totally hydrolyzed. In the Ag/Ab complex, the antigen substrate was blocked by antibody, which was also a substrate for the enzyme. In the Ag/dextran complex, DEAE-dextran was neither a substrate nor did it sterically block the enzyme. The experiments indicated that inhibition of enzyme hydrolysis of the protein antigen by antibody was competitive in nature.

Summary:

The kinetics of IgM and IgG antibody production in rabbits after primary and secondary immunization with BSA was determined by antigen binding analysis in sequential bleedings. HA titers of these sera were assessed by the binding method; there was no relationship to actual antibody content.

The protection of a protein antigen from enzyme hydrolysis by its specific antibody was found to be proportional to the binding capacity of IgG antibody in antiserum and seemed to be competitive in nature.

Publications:

None

LITERATURE CITED

1. U. S. Army Medical Unit. 1 July 1969. Annual Progress Report, FY 1969. p. 122 to 125. Fort Detrick, Maryland.

2. Farr, R. S. 1958. A quantitative immunochemical measure of the primary interaction between I^{131}BSA and antibody. J. Infect. Dis. 103:239-262.

3. Johnson, H. M., K. Brenner, and H. E. Hall. 1966. The use of a water-soluble carbodiimide as a coupling reagent in the passive hemagglutination test. J. Immun. 97:791-796.

4. McKernan, W. M., and C. R. Ricketts. 1960. A basic derivative of dextran and its interaction with serum albumin. Biochem. J. 76:117-120.

5. Fukazawa, Y., T. Shinoda, Y. Yomoda, and T. Tsuchiya. 1970. Antibody response to bacterial antigens: Characteristics of antibody response to somatic antigens of Salmonella typhimurium. Infection Immun. 1:219-225.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 02:	**Prevention and Treatment of Biological Warfare Casualties**
Work Unit No. 096 02 006:	**Evaluation of Humoral Factors Other Than Antibody in the Immune Response**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Benjamin B. Kirkland, Lt Colonel, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0892	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 05 06	D. CHANGE	U	U	NA	DE	XX YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	006
b. [illegible]				
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Evaluation of humoral factors other than antibody in the immune response

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 08	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	4
FISCAL YEAR CURRENT 71	1	5

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign intelligence considered.

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Immunity; (U) Immune mechanism; (U) Antibody inhibition; (U) Immunosuppression

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To develop a method to effect a specific immune inhibition.

24 (U) Rabbits are pretreated with seromucoid extracted from the antisera of immunized rabbits. The pretreated rabbits are then challenged with the same antigen; immune responses are compared to controls.

25 (U) 69 08 - 70 07 - An immune inhibition has been effected by pretreating rabbits with the acid soluble fraction of serum (seromucoid) extracted from rabbits immunized with bovine serum albumin (BSA). The inhibition appeared to be specific in that inhibition occurred when the pretreated rabbits were challenged with BSA, whereas no significant inhibition occurred in similarly pretreated rabbits challenged with another antigen, egg albumin. Also no inhibition occurred on pretreatment with seromucoid extracted from unimmunized rabbits. The seromucoid factor does not appear to be the antigen, BSA, or its antibody. Further studies are being done to characterize this factor.

*Available to contractors upon originator's approval.

DD FORM 1498 [illegible] (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 006: Evaluation of Humoral Factors Other Than Antibody in the Immune Response

Description:

Study the antibody response of animals following pretreatment with an acid soluble fraction derived from immune and nonimmune sera.

Progress:

Serum glycoproteins, particularly the α-fractions, are elevated in inflammatory and infectious diseases.[1] Although specific biological activity has not been ascribed to these fractions, there is evidence that α-glycoproteins from nonimmune animals have an immunosuppressive effect.[2] Therefore, an investigation was initiated to study whether glycoproteins from immunized animals would behave similarly.

In a preliminary study, 4 rabbits were hyperimmunized with bovine serum albumin (BSA) over a 10-week period. One week after the last injection, animals were exsanguinated and the sera were pooled. The seromucoid fraction was extracted by a modification of the method of Price, et al.[3] After precipitation with 0.48M $HClO_4$, the supernatant fluid was adjusted to pH 7.0 with KOH; the precipitate was discarded. After dialysis, the supernatant containing the seromucoid fraction was concentrated to one-fifth the original serum volume. Antibody activity was not demonstrable. Pooled serum from nonimmunized rabbits was treated in the same manner.

The effect of seromucoid on immune response was evaluated by pretreatment of a group of rabbits with immune seromucoid, and another with nonimmune seromucoid; a control group received no pretreatment. These preparations were administered by the intraperitoneal (IP) route. One half of each group was challenged with BSA and the other with egg albumin (EA). Antibody responses after challenge were measured by precipitation and hemagglutination techniques.

Results indicated that there was specific immune inhibition in rabbits challenged with BSA after pretreatment with the seromucoid fraction of rabbit anti-BSA sera. In contrast, significant inhibition did not occur in rabbits treated similarly but challenged with EA, or in animals pretreated with nonimmune seromucoid.

A second study was conducted to investigate those factors involved in inhibition of specific antibody production, and to confirm the preliminary data. Pooled sera were obtained from nonimmunized rabbits, from rabbits immunized with BSA in Freund's adjuvant, and from rabbits stimulated by Freund's adjuvant alone. Seromucoid fractions were obtained from each by precipitation with 0.26 M $HClO_4$ and further treated as described above.

Each seromucoid preparation was employed to pretreat a group of rabbits. Treatment consisted of 4 injections at weekly intervals; 1 ml was given IP and followed by 3 injections of 0.5 ml subcutaneously (SC). A control group received no pretreatment. Twenty-four hours following the 4th injection, a challenge sequence was initiated. This consisted of three 30-mg injections of BSA or EA at 3-day intervals. Approximately 3 weeks following the last injection, HA and precipitin titers were measured (Table I).

This study was complicated by death of animals from unexplained causes during treatment and challenge periods. Two of 3 survivors in the group given seromucoid derived from anti-BSA exhibited suppression of antibody response to BSA challenge; it is possible that antibody contained in this seromucoid preparation counteracted the suppressive effect in the 3rd animal. The disparate antibody responses in rabbits pretreated with Freund's seromucoid have not been explained. Seromucoid from nonimmune rabbits had no suppressive effect on antibody to BSA. Antigen specificity was evident because there was no suppression of response to challenge with EA. Studies are currently in progress to amplify these data.

The factor or factors responsible for specific inhibition by seromucoid have not as yet been identified. Seromucoid has been shown by electrophoresis to be a heterogeneous fraction of serum protein; α_1- and α_2-glycoproteins are predominant but a small amount of β-glycoprotein is normally present.[3] Characterization of our preparations has not been completed.

TABLE I. EFFECT OF PRETREATMENT WITH IMMUNE AND NONIMMUNE SEROMUCOID ON ANTIBODY RESPONSES OF RABBITS CHALLENGED WITH BSA AND EA

PRETREATMENT	CHALLENGED WITH	ANTIBODY RESPONSE	
		Reciprocal HA	Precipitin[a] µg antigen
Seromucoid extracted from sera of rabbits immunized with BSA in Freund's adjuvant	BSA	-	-
		-	-
		20	0.78
	EA	320	[b]
		160	
		320	
Seromucoid extracted from sera of normal (non-immunized) rabbits	BSA	10	3.12
		40	3.12
		20	0.78
		40	3.12
		40	3.12
Seromucoid extracted from sera of rabbits immunized with Freund's adjuvant alone	BSA	10	1.56
		-	-
		-	-
		20	6.25
		40	3.12
		-	-
	EA	40	
		320	
		40	
		40	
		20	
None	BSA	10	3.12
		40	1.56
		10	0.78
	EA	40	
		160	
		180	

a. Precipitated at equivalence by 1 ml of serum.

b. Final results not available; qualitative precipitin tests strongly positive.

Summary:

An acid soluble fraction (seromucoid) extracted from serum inhibited immune response. For the first time, inhibitory activity of seromucoid has been demonstrated to be specific for the original antigenic stimulus. Studies are continuing to extend these observations and define the factors involved in suppression of immune responses.

Publications:

None.

LITERATURE CITED

1. Heiskell, C. L., C. M. Carpenter, H. E. Weimer, and N. Schoichi. 1961. Serum glycoproteins in infectious and inflammatory diseases. Ann. N. Y. Acad. Sci. 94:183-209.

2. Mowbray, J. F. 1963. Ability of large doses of an alpha$_2$ plasma protein fraction to inhibit antibody production. Immunology 6:217-225.

3. Price, W. H., H. Harrison, and S. H. Ferebee. 1964. Disc electrophoresis on polyacrylamide gels of serum mucoids of individuals with selected chronic diseases. Ann. N. Y. Acad. Sci. 121:460-469.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 007:	Evaluation of Experimental Vaccines in Laboratory Animals
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Richard O. Spertzel, Major, VC J. Brent Rollins, Captain, VC Douglas W. Mason, Captain, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0893	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 05 06	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	007
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 12126(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Evaluation of experimental vaccines in laboratory animals

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	2	40
	CURRENT 71	2	40

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
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NAME: Rollins, J. B. DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Vaccines; (U) Immunization; (U) Staphylococcus; (U) Toxoid

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Evaluate experimental vaccines or antigens in laboratory animals before use in man.

24 (U) Promising vaccines are given to laboratory animals and safety tested prior to administration to man.

25 (U) 69 07 - 70 06 - The optimal immunizing schedule for the standard Pfizer lot of staphylococcal enterotoxin B (SEB) toxoid was found to be 2 doses of 50 micrograms antibody N 28 days apart. Five production lots of SEB toxoid were received. Two were tested in monkeys and found to be unsatisfactory, based upon criteria previously established for fever and emesis. One lot was tested for protective efficacy; it induced only low-grade immunity.

Acceptable fluid toxoid from Pfizer Co. was precipitated with alum. When tested for efficacy it was no better than the parent material.

A whole broth culture was toxoided with formalin for 7 days. Safety testing of the material produced fever and emesis typical of SEB toxemia.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 007: Evaluation of Experimental Vaccines in Laboratory Animals

Description:

Evaluate experimental vaccines or antigens in laboratory animals before use in man.

Progress, Part I:

To determine optimal dosage and schedule for administration of staphylococcal enterotoxin B (SEB) toxoid, a variety of immunizing schedules using Lot 87285 were tried. All groups were challenged with toxin 6 weeks after the last dose of toxoid was administered. Groups 1-10 were challenged with purified SEB (Lot 14-30). Groups 11-14 were challenged with 30% purity SEB (Lot 52-68); all animals were sick. The groups, along with dose, schedule and deaths, are shown in Table I.

No dose or schedule examined was any more or less effective than that (group 9) usually employed for toxoid evaluation. Lot 52-68 appeared to be more toxic than 14-30, but the toxoid still conferred some protection. Serologic studies are reported under Work Unit No. 096 03 800.

Five additional production lots of SEB toxoid produced by Charles P. Pfizer Co., Terre Haute, Ind. were received in November 1969. These 5 lots differed from Lot 87285 in that they were detoxified for only 30 days. One lot (96627) was standardized, filled, packaged and labeled exactly like Lot 87285; the remaining 4 lots were supplied in 100-ml amounts with several small-volume bottles from each lot for testing.

Results of safety tests (using the techniques previously reported[1/]) on 2 of these production lots (96627 and 4237J8) produced fever in 3 of 3 monkeys, and emesis in 2 of 3 monkeys. This does not meet the standards proposed for the finished product. Results of efficacy testing (using the standard technique) of Lot 96627 toxoid are shown in Table II. Fewer monkeys were protected against illness and lower precipitin titers developed, as compared to previously described lots.

TABLE I. RESPONSE[a] TO CHALLENGE AT 12 WEEKS OF MONKEYS IMMUNIZED WITH VARYING TOXOID SCHEDULES.

GROUP	μg TOXOID N GIVEN BY WEEKS							Total	% DEAD OF 6
	0	1	2	3	4	5	6		
	Challenged with Lot 14-30								
	300 μg/kg								
1			80				20	100	0
2	80						20	100	17
3	50						50	100	17
4	40	40					20	100	0[b]
5	40		40				20	100	0
8							100	100	17
9			50				50	100	0
6	50	50					50	150	0
7	50		50				50	150	17
10				Controls				0	50
	Challenged with Lot 52-68								
	10 μg/kg								
11			50				50	100	0
12				Controls				0	50
	300 μg/kg								
13			50				50	100	33
14				Controls				0	83

a. All monkeys sick except group 11, 4 of 6 sick.

b. Five monkeys only.

TABLE II. EFFICACY IN MONKEYS OF LOT 96627 SEB TOXOID AGAINST CHALLENGE WITH LOT 14-30 TOXIN

	10 μg/kg		300 μg/kg	
LOT 96627 TOXOID	Ill/Total	Dead/Total	Ill/Total	Dead/Total
Immunized	3/5	0/5	5/5	1/5
Controls	6/6	1/6	6/6	4/6

Summary, Part I:

The optimal immunizing schedule for Lot 87285 Pfizer toxoid appears to be 2 50-μg-N_2 doses 4 weeks apart. Two of the final 5 lots of Pfizer-produced toxoid did not meet safety standards proposed for this product; one lot tested for efficacy produced low-grade immunity.

Progress, Part II:

In an attempt to improve the immunogenicity of SEB toxoid, Pathology Division, USAMRIID, prepared an alum-precipitated toxoid from Lot 87285. Safety testing of this product using the technique described previously[1] proved that it was unsatisfactory. The quantity of alum present in the inoculum was sufficient to produce fever by itself. Efficacy tests using the standard schedule were performed.

Results in Table III show that the alum-precipitated toxoid was no better than the parent fluid toxoid (87285). Serological studies are reported under Work Unit No. 096 03 800.

TABLE III. EFFICACY IN MONKEYS OF ALUM-PRECIPITATED SEB TOXOID AGAINST CHALLENGE WITH LOT 14-30 TOXIN

	10 μg/kg		300 μg/kg	
TOXOID	Ill/Total	Dead/Total	Ill/Total	Dead/Total
Alum-87285	2/12	0/12	11/11	0/11
87285	1/12	0/12	12/12	1/12
Controls	12/12	2/12	12/12	10/12

Summary, Part II:

Alum-precipitated 87285 toxoid shows no advantage over the fluid toxoid.

Publications:

None.

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual Progress Report, FY 1969. p. 98. Fort Detrick, Maryland.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 008:	Evaluation of Efficacy of Combined Antigens in Man
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases
Divisions:	Medical and Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Peter J. Bartelloni, Lt Colonel, MC Robert W. McKinney, Lt Colonel, MSC Francis E. Cole, Jr., Ph.D. Helen H. Ramsburg, B.S.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0894	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 05 06	D. CHANGE	U	U	NA	DE	XX YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	008
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Evaluation of efficacy of combined antigens in man

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 02	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* NA

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	20
FISCAL YEAR CURRENT 71	2	20

19. RESPONSIBLE DOD ORGANIZATION

NAME:* USA Medical Research Institute of Infectious Diseases

ADDRESS:* Fort Detrick, Md 21701

RESPONSIBLE INDIVIDUAL

NAME: Crozier, D.

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20. PERFORMING ORGANIZATION

NAME:* Medical Division
USA Medical Research Institute of Infectious Diseases

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Francisco, N. M. DA

NAME:

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vaccines; (U) Immunization; (U) Encephalitis, equine (VEE, EEE, WEE)

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Test and evaluate combinations of vaccines in man.

24 (U) After combination antigens have been safety tested and evaluated in laboratory animals, they are given to man.

25 (U) 69 07 - 70 06 - A combined killed WEE + EEE vaccine was tested in man. Serum neutralization indices (SNI) to both components were significantly elevated (1.7 logs or greater) by day 42, remaining so through day 270. Both had slight decreases in mean values at 1 year.

Combined WEE + EEE was given simultaneously with live attenuated VEE vaccine to 16 volunteers. Clinical reactions were mild to moderate. The mean SNI to EEE antigen was 1.7 logs or greater by day 28 and to WEE and VEE by day 42. Significant mean levels for all 3 persisted through day 90. Six, 9 and 12 month data are not yet available.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 008: Evaluation of Efficacy of Combined Antigens in Man

Description:

Test and evaluate combinations of vaccines in man.

Progress, Part I:

Evaluation of Combined Western Equine Encephalitis (WEE) Vaccine, Lot 1-1967, and Eastern Equine Encephalitis (EEE) Vaccine, Lot 1-1966, in Volunteers (Project No. FY 69-6): Clinical responses and laboratory determinations on 16 volunteers administered combined, inactivated WEE and EEE vaccines were reported previously.[1] Neutralization tests on blood obtained over a 1-year period have been completed.

The majority of subjects failed to achieve significant WEE neutralizing antibody titers 14 and 28 days after the first dose of combined vaccine (Table I). The mean WEE serum neutralization index (SNI) on days 14 and 28 was 1.4 logs. Fourteen days after the second dose of vaccine (day 42) the mean was 1.7 logs although 8 of 16 subjects had a SNI < 1.6 logs. The mean WEE SNI remained at acceptable but low levels through day 270. However, by day 360 the mean SNI was 1.6. Moreover, 4 of 16 subjects did not achieve significant WEE neutralizing antibody titers throughout the entire study and by day 360 only 6 of 16 subjects had significant neutralizing antibody titers to WEE virus.

The majority of subjects failed to attain significant EEE SNI (Table II) by 14 days after the first dose of combined vaccine. The mean SNI 28 days after the first dose of combined vaccine was 1.7 logs. By day 42 the mean SNI was 2.7, 100% having antibody titers ≥ 1.7 logs. The mean EEE SNI remained at acceptable levels through day 270, with a decrease in mean on day 360 to 1.5 logs, when only 6 of 16 subjects had titers ≥ 1.7 logs. A decrease in EEE neutralizing antibody titer occurred on day 90 in 1 subject, day 180 in 4 subjects and on day 360 in 5.

TABLE I. WEE VIRUS NEUTRALIZING ANTIBODY RESPONSES[a] OF VOLUNTEERS ADMINISTERED 2 DOSES OF COMBINED WEE AND EEE VACCINES ON DAYS 0 AND 28

VOLUNTEERS	LOG_{10} SNI BY DAY POSTVACCINATION							
	14	28	42	56	90	180	270	360
WPB	0.8	1.0	1.4	1.8	1.8	1.4	2.0	1.4
GLB	2.5[b]	2.2	2.0	2.5	2.5	2.5	2.8	2.3
RBB	1.3	1.3	1.8	2.2	2.3	1.6	1.6	1.6
LNC	2.3	2.8	3.3	3.3	3.1	2.6	2.9	2.4
DDC	0.4	0.3	0.5	1.1	0.3	0	0.2	0
JBC	1.4	1.4	0.8	0.8	0.8	0.8	0.8	0.3
HDG	1.0	1.4	0.5	0.5	0.5	0.3	1.0	1.3
DEG	0.9	1.0	1.1	0.8	0.5	0.2	0.1	0.2
KKH	1.1	1.1	2.0	2.0	1.9	1.7	1.9	1.5
DCH	1.3	1.6	2.0	2.3	2.3	2.4	3.1	2.3
RML	1.1	1.3	2.3	2.2	2.1	2.0	2.1	2.1
REL	2.8	3.3	3.4	3.3	3.4	3.8	3.8	3.3
RWO	1.4	1.4	2.3	2.2	2.2	1.7	1.3	1.5
REP	0	0.6	0.9	1.4	1.4	1.9	1.3	1.6
JDS	ND[c]	0.4	1.0	1.2	1.2	1.6	1.8	1.4
KGW	1.7	1.5	1.6	2.3	2.1	2.8	2.5	3.0
No. $\geq$ 1.7 logs	4	3	8	10	10	9	9	6
Mean	1.4	1.4	1.7	1.9	1.7	1.7	1.8	1.6
Range	(0.4-2.5)	(0.3-3.3)	(0.5-3.4)	(0.5-3.3)	(0.3-3.4)	(0-3.8)	(0.1-3.8)	(0-3.3)

a. No subjects had demonstrable neutralizing antibody on day 0.
b. ___ = $\geq$ 1.7 logs.
c. ND = not done.

TABLE II. EEE VIRUS NEUTRALIZING ANTIBODY RESPONSES[a] OF VOLUNTEERS ADMINISTERED 2 DOSES OF COMBINED WEE AND EEE VACCINES ON DAYS 0 AND 28

VOLUNTEERS	LOG_{10} SNI BY DAY POSTVACCINATION							
	14	28	42	56	90	180	270	360
WPB	1.1	1.8	2.0	1.9	1.3	1.4	1.5	1.9
GLB	1.6	1.7	2.8	3.0	2.6	2.5	1.7	1.8
RBB	0.8	1.0	2.5	2.2	1.9	1.8	2.1	1.6
LNC	1.0	1.9	2.6	2.2	2.0	2.3	2.1	1.1
DDC	1.9[b]	1.5	2.6	2.3	2.3	1.1	1.1	1.1
JBC	1.2	1.4	2.3	2.0	1.4	1.5	0.5	0.9
HDG	0.8	1.0	2.1	2.3	2.1	1.6	1.9	1.0
DEG	2.0	2.6	3.0	2.7	2.6	1.9	1.5	2.0
KKH	2.3	1.5	3.1	2.3	1.8	1.7	2.0	1.5
DCH	1.6	1.7	2.9	2.8	2.5	2.1	2.1	1.2
RML	1.5	2.1	3.5	3.8	2.8	1.4	1.4	1.6
REL	0.8	0.8	2.8	1.9	1.8	2.2	2.1	1.8
RWO	2.4	2.5	3.7	3.1	3.0	1.4	1.8	1.3
REP	0.8	1.5	2.3	2.2	1.9	1.1	1.5	1.1
JDS	ND[c]	1.4	2.3	2.8	2.4	2.5	2.7	2.4
KGW	1.6	2.1	3.3	2.7	2.7	1.6	2.2	2.0
No. $\geq$ 1.7 logs	4	8	16	16	14	8	10	6
Mean	1.5	1.7	2.7	2.5	2.2	1.8	1.8	1.5
Range	(0.8-2.4)	(0.8-2.6)	(2.0-3.7)	(1.9-3.8)	(1.3-3.0)	(1.1-2.5)	(1.1-2.7)	(0.9-2.4)

a. No subjects had demonstrable neutralizing antibody on day 0.
b. ___ = $\geq$ 1.7 logs.
c. ND = not done.

Summary, Part I:

A combined Western and Eastern inactivated vaccine was evaluated in man. A significant mean WEE SNI was not achieved until day 42, 14 days after the administration of the second dose of vaccine. SNI remained significant through day 270. Four of 16 subjects failed to attain significant SNI throughout the study period; on day 360 only 6 of 16 sugjects had significant SNI. By day 42 the mean EEE SNI was 2.7 logs and all subjects had achieved significant titers. However, by day 360 the mean SNI had decreased to 1.5 logs and only 6 of 16 subjects had significant EEE neutralizing antibody titers.

Progress, Part II:

Evaluation of Combined WEE Vaccine, Lot 1-1967, and EEE Vaccine, Lot 1-1966, Administered Simultaneously with Venezuelan Equine Encephalomyelitis (VEE) Vaccine, Attenuated, NDBR 102-2 (Project No. FY 69-8): Clinical and serological responses were evaluated in 16 volunteers administered combined WEE and EEE vaccine given simultaneously with VEE vaccine. The WEE and EEE vaccines were reconstituted and combined as described previously.[1/] Each 0.5 ml of combined product contained approximately 100 hamster intraperitoneal median immunizing doses ($IPID_{50}$) of each vaccine. Each 0.5 ml of reconstituted VEE vaccine contained the standard human dose of approximately $10^{3.7}$ guinea pig $IPID_{50}$. The combined WEE and EEE vaccine and the VEE vaccine were inoculated in 0.5-ml amounts subcutaneously in different arms. Twenty-eight days later a second 0.5 ml dose of combined WEE and EEE vaccine was administered. Four volunteers served as controls and received 0.5 ml Isotonic Sodium Chloride for Injection, Sterile, U.S.P. subcutaneously. Each subject had frequent laboratory determinations including white blood cell and differential counts, hematocrits, quantitative platelet counts, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, direct and indirect serum bilirubin, blood urea nitrogen, alkaline phosphatase, and urinalysis; in addition daily electrocardiograms were performed. All vaccinees were observed for 12 days for local and systemic reactions. Following the administration of the second dose of combined WEE and EEE vaccine, subjects were observed at 24 and 48 hr postvaccination on an outpatient basis. Blood was drawn to obtain serum for mouse neutralization tests prior to immunization and on days 14, 28, 42, 56, 90, 180, 270, and 360.

Systemic and local reactions are shown in Table III. Two subjects had minimal local reactions on day 0, whereas 10 had systemic reactions the majority of which were minimal. Following the second dose of combined inactivated WEE and EEE vaccine 4 had minimal local reactions while 10 had systemic reactions which varied from minimal to mild. No serious reactions were observed.

TABLE III. REACTOGENICITY TO COMBINED WEE + EEE (0.5 ml) AND VEE (0.5 ml) VACCINES[a] IN 16 NONEXPERIENCED SUBJECTS

REACTION	(DAY)	SEVERITY[b]			
		None	Minimal	Mild	Moderate
Local	(0)	14	2		
	(28)	12	4		
Systemic	(0)	6	6	2	2
	(28)	5	6	4	1

a. WEE + EEE and VEE vaccines given day 0, second dose of WEE + EEE given on day 28.

b. Local reactions:
Minimal = discomfort with or without tenderness.
Mild = erythema with or without induration.
Moderate = marked swelling, fluctuation.
Severe = necrosis.
Systemic reactions:
Minimal = Subjective complaints, < 48 hr duration.
Mild = Subjective complaints, body temperature 100-101 F.
Moderate = Subjective complaints, temperature 101-102 F, ambulatory.
Severe = Subjective complaints, temperature > 102 F, not ambulatory.

No meaningful changes in clinical laboratory values were observed during the period of study. Serum neutralization tests for each virus have been completed on sera collected through day 90; these results are shown in Table IV. Most subjects (12 of 16) failed to achieve a significant SNI to WEE virus until 14 days after the administration of the second dose of combined vaccine (day 42) at which time the mean SNI was 2.1 logs; comparable levels persisted through day 90. The mean EEE SNI 28 days after the initial dose of combined vaccine was 2.0 logs, with 4 subjects remaining at $\leq$ 1.6 logs. On days 42, 56 and 90 all 16 had titers $\geq$ 1.7 logs. By day 28 the mean SNI to VEE virus was 2.4 logs, 14 of 16 being $\geq$ 1.7 logs; similar high titers persisted through day 90.

Table V presents data from several studies for comparative purposes. It can be seen that WEE responses were delayed slightly in the combined vaccine studies when compared to WEE vaccine alone. Responses to the EEE component was essentially the same among the 3. Concerning VEE, it is not possible to compare these data with those of previous studies, since neutralization tests were not routinely performed on earlier volunteer studies with this vaccine.

TABLE IV. SEROLOGICAL RESPONSES[a] OF VOLUNTEERS ADMINISTERED COMBINED EEE + WEE VACCINE SIMULTANEOUSLY WITH LIVE VEE VACCINE ON DAY 0 AND SECOND EEE + WEE ON DAY 28

VOLUNTEERS	LOG_{10} SNI OF SERA TESTED WITH VIRUS INDICATED BY DAY POSTVACCINATION											
	28			42			56			90		
	WEE	EEE	VEE	WEE	EEE	VEE	WEE	EEE	VEE	WEE	EEE	VEE
JLC	0	1.9[b]	1.9	1.9	2.1	2.4	2.0	2.6	2.5	2.9	2.8	3.1
CGD	0.3	1.4	2.2	1.6	2.3	2.3	2.2	2.6	2.3	2.4	2.1	2.5
JED	0.4	1.5	3.0	1.3	2.8	3.0	1.5	2.5	3.0	1.8	2.3	3.0
KJF	0.6	2.4	2.5	2.1	3.0	3.0	2.3	3.0	3.0	2.2	3.0	3.6
REH	0.3	2.0	2.0	1.8	3.0	2.9	2.6	2.5	2.1	2.8	1.7	2.4
MWM	0.1	2.3	1.6	1.8	3.0	1.2	2.2	2.8	2.1	2.6	2.4	1.7
JDM	0.3	2.0	2.3	1.9	2.6	2.1	2.2	3.3	2.2	2.3	2.6	2.9
DLM	0.1	2.6	1.4	1.9	3.3	2.0	2.9	2.9	2.2	2.1	2.7	2.3
CAM	0.3	2.0	2.0	2.7	3.0	2.6	3.0	2.8	2.7	2.2	3.2	2.8
MDN	0.1	1.5	2.6	1.6	2.6	2.9	2.1	2.9	2.8	2.1	2.4	3.0
MCR	0.3	2.3	2.8	2.2	3.0	3.0	2.2	2.0	3.5	2.7	2.2	3.0
LBR	0	2.1	2.9	2.0	3.2	2.9	2.0	2.1	2.9	1.6	2.8	2.8
GLR	0	3.0	1.8	1.6	3.0	2.5	2.2	3.8	2.5	2.4	3.0	1.8
KMR	1.3	2.0	1.9	4.0	3.4	1.8	3.7	2.9	1.8	3.6	2.0	2.2
DRS	0	1.6	2.9	2.3	2.4	3.1	3.1	2.2	3.0	3.0	2.5	3.1
DFL	1.0	1.5	2.9	2.0	2.6	3.0	2.0	2.5	3.1	2.0	1.8	3.4
No. $\geq$ 1.7 logs	0	10	14	12	16	15	15	16	16	15	16	16
Mean	0.3	2.0	2.4	2.1	2.8	2.5	2.4	2.7	2.6	2.4	2.5	2.7
Range	(0-1.3)	(1.4-3)	(1.4-3)	(2.1-3.3)	(1.3-4)	(1.2-3.1)	(1.5-3.7)	(2.1-3.8)	(1.8-3.5)	(1.6-3.6)	(1.7-3.2)	(1.7-3.6)

a. No subjects had demonstrable neutralizing antibody on day 0.
b. ___ = $\geq$ 1.7 logs.

TABLE V. COMPARISON OF MEAN TITERS OF MEN IMMUNIZED WITH WEE AND EEE COMBINED VACCINES

STUDY	NO. MEN	% WITH SNI ≥ 1.7 LOGS BY DAY POSTVACCINATION							
		14	28	42	56	90	180	270	360
		WEE TITERS							
69-3, -4[a] WEE alone	21	14	48	100	95	90	95	90	95[b]
	Mean SNI	1.4	1.6	2.5	2.4	2.3	2.3	2.4	2.3
69-6[c] WEE + EEE	16	27[b]	19	50	84	84	56	56	38
	Mean SNI	1.4	1.4	1.7	1.9	1.7	1.7	1.8	1.6
69-8 WEE + EEE and VEE	16		0	75	94	94			
	Mean SNI		0.3	2.1	2.4	2.4			
		EEE TITERS							
68-2 EEE alone	8	13	38	88	100	ND[d]			
	Mean SNI	1.3	1.6	2.2	2.3				
69-6 WEE + EEE	16	27[b]	50	100	100	88	50	63	38
	Mean SNI	1.5	1.7	2.7	2.5	2.2	1.8	1.8	1.5
69-8 WEE + EEE and VEE	16		69	100	100	100			
	Mean SNI		2.0	2.8	2.7	2.5			

a. Three dosage schedules combined, see Work Unit 096 02 002.
b. One not done.
c. Part I, this work unit.
d. Not done.

Summary, Part II:

Sixteen volunteers were administered 2 doses of 0.5 ml of combined WEE + EEE vaccine 28 days apart and a single standard dose of VEE vaccine. Clinical reactions to the vaccines varied from minimal to moderate with only 2 subjects developing moderate systemic reactions. The immunogenicity of the composite vaccine was demonstrated by the achievement of significant mean SNI to EEE and VEE virus 28 days after the initial dose of vaccine and persistence of mean titers through day 90. All subjects developed significant SNI by day 42 to EEE and VEE viruses. Significant mean SNI to WEE virus were attained by day 42. Fifteen of 16 volunteers acquired meaningful WEE SNI by day 56 which persisted through day 90.

Publications:

None.

LITERATURE CITED

1. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual progress report, FY 1969. p. 87 to 102. Fort Detrick, Maryland.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 102:	Development and Evaluation of an Effective Vaccine Against Pneumonic Plague
Reporting Installation:	U.S. Army Medical Research Institute of Infectious Diseases Forest Glen Section Washington, D.C.
Division:	Microbiology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	John D. Marshall, Jr., Colonel, MSC (I, II) William H. Habig, Captain, MSC (III) Daniel N. Harrison, M.S. (II) Dan C. Cavanaugh, Lt Colonel, MSC (WRAIR) (I,IV) James H. Rust, Jr., Ph.D. (WRAIR) (IV)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0833	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	102
b. [illegible]				
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Development and evaluation of an effective vaccine against pneumonic plague

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 12	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	3	85
FISCAL YEAR CURRENT 71	3	85

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede each with Security Classification Code)
(U) Pasteurella pestis; (U) Plague; (U) Pneumonic plague vaccine; (U) Antigens; (U) Immunization; (U) Cytochrome

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Determine the factors influencing the susceptibility to plague infection and the most appropriate method to prevent the infection.

24 (U) Using standard methods, strains of Pasteurella pestis are tested for their physiological and virulence characteristics. Serological tests are performed on wild and laboratory animals for the detection of anti-P. pestis Fraction I antibody.

25 (U) 69 07 - 70 06 - Temperature is the most critical condition influencing the fibrinolysin test for P. pestis. Sera from various species of animals differ in their ability to inhibit the lysis of a standard bovine fibrin clot by P. pestis. Serum from known resistant species inhibit the reaction whereas sera from susceptible species do not. Man falls intermediate between these 2 groups. Strains of P. pestis isolated in the Republic of Vietnam during 1968-1970 were similar to strains isolated during previous years. The apparent decline in the prevalence of human plague in certain well defined foci cannot be attributed to an alteration in the causative organisms.

The occurrence of a pseudolysogenic strain of P. pestis was observed. Two b-type cytochromes have been found in aerobically grown P. pestis. One of these, cytochrome o, has been highly purified and characterized spectrally, physically, and immunologically. Preliminary data indicate that this antigen may be useful in the serologic distinction between immunization and true infection.

It has been demonstrated that laboratory rats and mice are highly susceptible to infection with P. pestis when challenged orally with infected carcasses. Dogs and cats appear less susceptible than rats although cats are somewhat more susceptible than dogs. All surviving dogs and cats had demonstrable specific plague antibodies.

Publications: Bull. Wildlife Dis. Ass. 5:187-194, 1969; In Manual of Clinical Microbiology, 1970, p. 205-209; In Plague, 1970, In press.

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B622706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 102: Development and Evaluation of an Effective Vaccine Against Pneumonic Plague

Description:

Determine the factors influencing the susceptibility to plague infection and the most appropriate method to prevent the infection.

Progress, Part I:

Differences in natural susceptibility to plague among various animal species have been recognized since the Manchurian plague epidemic of 1920. More recently 1 of the virulence factors, Pesticin I, fibrinolysin, coagulase complex (PFC) has been shown to be related to invasiveness of Pasteurella pestis.[1] The action of P. pestis fibrinolytic factor has been shown to be similar to that of staphylokinase and urokinase, directly activating plasminogen which then lysed the fibrin clot.[2] Madison[3] reported that P. pestis differed in its ability to lyse the clots prepared from plasma of different species of animals. Contrary to these results, Domaradskii and Yarmyuk[4] reported that clots prepared from purified fibrin of various species of animals were equally susceptible to the action of P. pestis fibrinolytic factor. A series of experiments were conducted to clarify the apparent discrepancies of these findings. A fibrinolysin test using purified bovine fibrin clot, acetone killed and dried P. pestis was standardized. The clot lysis inhibition of sera of various animal species was determined.

During the standardization procedure it was found that the temperature of the reaction was the most critical factor. At temperatures below 27 C the reaction time was prolonged and required 5 - 20 times the concentration of P. pestis cells to cause complete lysis at temperature of $\geq$ 30 C. This data was found to correlate with flea transmission data, and to be in agreement with observations of spontaneous decline in the epidemic curve of human plague in Vietnam and elsewhere.

When the sera of various animal species were tested for their ability to inhibit the lysis of a standard bovine fibrin clot by P. pestis, it was observed that the sera of highly susceptible species failed to inhibit clot lysis. The sera of known resistant species were found to inhibit the reaction to various degrees. Human sera were found to be intermediate between the highly susceptible and the highly resistant groups. The presence or absence of P. pestis Fraction I antibody did not appear to influence the results of the test with either human or animal sera.

Summary, Part I:

Temperature is the most critical condition influencing the P. pestis fibrinolysin test. Sera from various species of animals differ in their ability to inhibit the lysis of a standard bovine fibrin clot by P. pestis. Serum from resistant species inhibit the reaction whereas sera from susceptible species do not. Man falls intermediate between these 2 groups.

Progress, Part II:

During the reporting period, an additional 350 cultures of P. pestis isolated in Vietnam were characterized according to criteria reported last year.[5] Strains isolated from areas where the human disease was on the decline were in no way different from strains isolated from the same areas during previous years of peak epidemic activity. Therefore, the apparent burn-out of these focal epidemics cannot be attributed to alteration in virulence of P. pestis as suggested by earlier workers.[6]

A strain of P. pestis was found to be pseudolysogenic. While this condition has been induced by laboratory manipulation[7], it has not been reported as a naturally occurring state in strains isolated directly from humans. The culture as received from Vietnam contained a mixed population of 3 biotypes of P. pestis and a phage which differed in its properties from the standard typing phage used in Vietnam. Eighty per cent of the population of P. pestis cells in the culture were resistant to lysis by either phage. These biotypes have been characterized; all are of reduced virulence due to loss of the pigmentation factor. The newly isolated phage, while serologically related to the standard one, is more active against a small number of laboratory strains selected for phage resistance. The new P. pestis phage has been incorporated into the routine testing procedure.

Summary, Part II:

Strains of P. pestis isolated in the Republic of Vietnam during 1968-1970 were similar to strains isolated during previous years. The apparent decline in the prevalence of human plague in certain well defined foci cannot be attributed to an alteration in the causitive organism. The occurrence of a pseudolysogenic strain of P. pestis was observed.

Progress, Part III:

The immune status of an individual with respect to plague is frequently evaluated serologically by means of the hemagglutination (HA) test with Fraction I protein, the capsular antigen of P. pestis. However, in the course of studies of nonencapsulated strains as vaccines, it became necessary to obtain an alternative purified antigen common to both wild-type and nonencapsulated bacteria. A reddish-brown antigenic protein was isolated and later identified as cytochrome o (a bacterial terminal oxidase with a b-type or protoheme prosthetic group).

Cytochrome o was purified from sonic extracts of aerobically grown P. pestis, strain M23NP, by DEAE cellulose chromatography and gel filtration. Material prepared in this manner was shown to be homogeneous by disc electrophoresis, gel filtration, ultracentrifugation, and immunologic techniques. Cytochrome o has a sedimentation constant of 8.7S, a MW of approximately 175,000 and an isoelectric point of 6.8. This cytochrome o has been carefully characterized spectrally, is auto-oxidizable, forms a complex with CO characteristic of bacterial oxidases, and possesses a protoheme-type alkaline pyridine ferrohemochrome spectrum. Attempts to determine the redox potential have not yet been successful due to failure of common redox dyes to couple with the purified protein. This problem has also prevented in vitro analysis of the purified cytochrome by oxygen uptake studies. Washed particulate fractions of sonicated bacterial cells have, however, demonstrated vigorous oxygen uptake in the presence of $DPNH_2$ or succinate. Oxygen uptake could be stimulated 50% by addition of purified cytochrome o to such a system. Respiratory activity of those particles as studied by oxygen uptake and spectral methods indicates insensitivity to inhibitors such as rotenone, azide and anti-mycin A.

In addition to the oxidase, another b-type cytochrome was detected. This cytochrome was not auto-oxidizable, formed no complex with CO, and possessed spectral and physical properties distinct from those of the oxidase. Alkaline pyridine ferrohemochrome spectra of whole cell extracts revealed only protoheme. Difference spectra at -196 C also revealed only b-type cytochromes. In P. pestis, therefore, only 2 cytochromes have been detected. This is a unique observation and may represent a primitive evolutionary form of the electron transport chain.

P. pestis cytochrome o is highly antigenic. Precipitating, HA and complement fixing antibodies are readily elicited in rabbits by cytochrome o injection, with or without the use of adjuvant. Such antibody reveals only 1 line by Ouchterlony immunodiffusion against either the homologous antigen or the crude P. pestis soluble protein preparation. Mice, injected with 2 doses of varying quantities of cytochrome o, were not protected against challenge with 1,000 median lethal doses of P. pestis, strain 195P.

Preliminary evidence suggests that animals surviving challenge frequently have an anti-cytochrome o titer, whereas animals immunized with killed vaccine seldom do. This observation is being investigated further to develop a method of distinguishing retrospectively between immunization and true infection. Such a method, if available, would have great potential value in epidemiological studies.

Cytochrome o isolated from Pasteurella pseudotuberculosis has been compared to the oxidase of P. pestis by a quantitative precipitin technique. The results indicate they are identical. These studies are being extended to other species of the Pasteurellae in hopes of clarifying their taxonomy.

Summary, Part III:

Two b-type cytochromes have been found in aerobically grown P. pestis. One of these, cytochrome o, has been highly purified and characterized spectrally, physically, and immunologically. Preliminary data indicate that this antigen may be useful in the serologic distinction between immunization and true infection.

Progress, Part IV:

The value of serological methods in the ecology and epidemiology of plague has received a great deal of attention in the past several years. In order to evaluate the significance of the results obtained in serological surveys, laboratory experiments were designed to examine the time of appearance of antibody following subclinical and clinical plague infection in animals as well as the type of antibody produced. Ancillary studies were carried out on the role of cannibalism in the maintenance of plague infection in rodent populations when the flea population is not of sufficient density to maintain the disease in rodent populations. Experiments on cannibalism performed in the past were not consistently reproducible and thus were not assumed to be of importance in the ecology of plague[6].

Experiments carried out in our laboratory indicate that fatal plague infection consistently occurs in rodents feeding on carcasses of animals that have died from plague infection. The disease pattern of animals challenged in this way is similar to that in animals challenged by bites of

infected fleas. The gross pathology of animals infected by ingestion of diseased carcasses, however, varies. Lung involvement appears more frequently in the form of a pneumonic process with massive infiltration and occasional hemorrhage. Animals infected orally also show enlarged mesenteric nodes as well as cervical and other peripheral lymphadenopathy similar to animals infected by subcutaneous and IP routes. The mean time of death of these orally infected animals is 2.6 days and is 100% fatal. Attempts to induce infection with in vitro cultured organisms have not been uniformly effective. Laboratory chow has been impregnated with organisms grown at 25 C and at 37 C and fed to rats. Death occurred in all animals fed with 25 C-grown organisms, but occurred only occasionally with the 37 C-grown bacteria. Further experiments indicated that by utilizing a 24-hr culture of organisms grown at 37 C death of the animals usually occurred. Cultures maintained at 37 C for > 24 hr rarely killed the animals even though it could be demonstrated that the population of bacteria utilized did not vary in the proportion of VW-positive organisms present. Antibody could not be demonstrated in any of the surviving animals and all succumbed to challenge with virulent organisms.

Immunized animals were protected against oral challenge to the same extent as those challenged by the subcutaneous or IP route. Animals that had been fed carcasses of mice infected with the vaccine strain EV-76 showed evidence of subclinical infection by demonstrating the formation of measurable antibody and by survival following infection with fully virulent challenge.

A modified mouse epizootic was established in a mouse colony free of the vector flea by the introduction of an infected mouse carcass; by replacing the animals that died, it was maintained for > 30 days. All animals that died were shown by culture and pathological alterations to have died from overt plague disease.

Since large predator animals from plague endemic areas frequently have been shown to have antibody to the capsular material of the plague bacillus experiments were designed to test the susceptibility of dogs and cats to both oral and parenteral challenge and to measure the antibody produced following such challenge. It was shown that cats could be infected by ingesting infected mice and by inoculation with virulent organisms. Two of 3 cats died following ingestion of a single plague infected mouse while 1 of 2 died following subcutaneous inoculation. Five dogs survived (2 challenged subcutaneously and 3 challenged orally by feeding a single infected mouse). The 2 surviving cats showed evidence of disease in that they were febrile for several days and organisms could be obtained from blood and in 1 instance from pleural fluid. The disease was evident in only 2 dogs which were febrile for a few days.

All of the survivors, both dogs and cats, demonstrated antibody between days 9 and 12 infection. Studies are currently in progress to establish the localization of the antibody formed as to its occurrence in the smaller 7S or macro 19S γ globulin, as well as the persistence of the antibody with respect to time.

Summary, Part IV:

It has been demonstrated that laboratory rats and mice are highly susceptible to infection with P. pestis when challenged orally with infected carcasses. Dogs and cats appear less susceptible than rats although cats are somewhat more susceptible than dogs. All of the surviving dogs and cats had demonstrable specific plague antibody following challenge. Immunization of rats was observed following ingestion of animal carcasses infected with avirulent P. pestis. This immunization was demonstrated by serological methods as well as survival following either oral or subcutaneous challenge with highly virulent organisms. Attempts to measure the time of appearance of the different antibody globulins and the persistence of such globulins is currently in progress.

Presentations:

1. Marshall, J.D., Jr. Plague. Presented at the Global Medicine Course, Walter Reed Army Institute of Research, 8 July 1969, and 17 February 1970.

2. Marshall, J.D., Jr. Relationship between diseases of wild and domestic animals. Model 2--Pasteurellosis. Presented at the National Animal Disease Laboratory, U.S. Dept. of Agriculture, Ames, Iowa, 16-18 June 1969.

Publications:

1. Cavanaugh, D.C., P.F. Ryan, and J.D. Marshall, Jr. 1969. The role of commensal rodents and their ectoparasites in the ecology and transmission of plague in Southeast Asia. Bull. Wildlife Dis. Ass. 5:187-194.

2. Marshall, J.D., Jr. and D.C. Cavanaugh. 1970. Pasteurella, p.205 to 209. In J.E. Blair. Manual of Clinical Microbiology, (ed.) American Society for Microbiology, Bethesda, Maryland.

3. Winter, P.E., D.C. Cavanaugh, J.D. Marshall, Jr., and J.H. Rust, Jr. 1970. Plague, In B. MacGraith, (ed.) Textbook on Tropical Disease. In press.

LITERATURE CITED

1. Brubaker, R.R., M.J. Surgalla, and E.D. Beesley. 1965. Pesticinogeny and bacterial virulence. Zbl. Bakt.(Naturwiss) Abt. I. Orig. 196:302-312.

2. Beesley, E.D., R.R. Brubaker, W.A. Janssen, and M.J. Surgalla. 1967. Pesticins: III. Expression of coagulase and mechanisms of fibrinolysis. J. Bact. 94:19-26.

3. Madison, R.R. 1936. Fibrinolytic specificity of Bacillus pestis. Proc. Soc. Exp. Biol. Med. 34:301-302.

4. Domaradskii, I.V., and G.A. Yarmyuk. 1960. Lysis of human and animal fibrins by plague microbe. Bull. Exptl. Biol. Med. (USSR), English translation, 56:55-58.

5. U.S. Army Medical Research Institute of Infectious Diseases, 1 July 1969. Annual Progress Report, FY1969, p. 127 to 132. Fort Detrick, Maryland.

6. Pollitzer, R. 1954. Plague, World Health Organization, Geneva.

7. Girard, G. 1957. Characters of pseudolysogenic mutants derived from P. pestis by bacteriophages. C.R. Soc. Biol. (Paris) 151:1068-1071.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 300:	Immunologic Studies with Rickettsiae
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Marie L. Miesse, B.S. (I) David M. Robinson, Major, VC (II)
Reports Control Symbol:	RCS-MEDDH-228(R1)
Security Classification:	UNCLASSIFIED

100 JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0885	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	300
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Immunologic studies with rickettsiae.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: NA d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	2	8
	CURRENT 71	2	8

19. RESPONSIBLE DOD ORGANIZATION

NAME: USA Medical Research Institute of Infectious Diseases
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20. PERFORMING ORGANIZATION

NAME: Virology Division
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TELEPHONE: 301 663-4111 Ext 7241
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Robinson, D. M.
NAME: DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Immunology; (U) Rickettsial diseases; (U) Spotted fevers; (U) Vaccines; (U) Q fever; (U) Coxiella burneti

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop vaccines of low reactogenicity for immunoprophylaxis against specific rickettsial diseases.

24 (U) Propagate representative strains in tissue culture systems. Assess the feasibility of producing rickettsial suspensions of quality and quantity suitable for vaccines for human use.

25 (U) 69 07 - 70 06 - No studies were conducted on the spotted fever rickettsiae during the year.

A master seed lot of Coxiella burneti (M44-WR-E1) and a vaccine lot (M44-WR-E2) were produced and sterility tested at the Department of Biologics Research, WRAIR. Characterization of M44-WR-E2 by us has indicated that while this strain is infectious for guinea pigs it is nonlethal for this host in contrast to the common phase I and phase II strains. Guinea pigs serologically converted after infection with M44 strain were resistant to challenge with virulent strain EP88. The challenge endpoint was equal to the complement fixation endpoint, which was 2.5 log greater then the febrile endpoint. Tetracycline and oxytetracyline at 1 and 5 times the human dosage levels were effective in reducing the febrile period, but one-fifth the human dose was not. Chloramphenicol was not as effective.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662702A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 300: Immunologic Studies with Rickettsiae

Description:

Develop vaccines of low reactogenicity for immunoprophylaxis against specific rickettsial diseases.

Progress and Summary Part I:

No studies were conducted during this report period with the spotted fever group of rickettsia due to delays in the useful occupancy of the new USAMRIID Laboratories. Studies will be resumed when facilities become available.

Progress Part II:

Investigations were begun on the M44 vaccine strain of Coxiella burneti.[1/] A master seed lot (Lot M44-WR-E1) produced from the original material, and a lot of vaccine (Lot M44-WR-E2) produced as first passage material from the master seed were obtained from the Department of Biologics Research, WRAIR. All materials were prepared as 20% yolk-sac suspensions in Snyder's I buffer and filled in 1-ml aliquots. The master seed lot was stored as fluid at -60 C. The vaccine lot was divided, with suitable amounts being freeze-dried or left as fluid; both were stored at -60 C. These materials were tested for sterility at WRAIR in accordance with Public Health Service Regulations for vaccines for human use.

On intraperitoneal (IP) titration in guinea pigs Lot WR-E2 had a titer of $10^{4.3}$ mean febrile doses (FD_{50}) per ml. Fever was taken to be temperatures $\geq$ 104 F. All guinea pigs given $10^{4.0}$ or $10^{3.0}$ FD_{50} IP were febrile on day 2, and afebrile by day 7. Guinea pigs inoculated with 10^{2} FD_{50} IP had a delayed onset of fever (day 3) but were afebrile at the same time as animals inoculated with 1 or 2 logs more rickettsia. The intensity of the febrile reaction was the same regardless of dilution employed, i.e., 85-100% had temperatures $\geq$ 105 F. In contrast guinea pigs inoculated IP with 10 FD_{50} had a prolonged incubation period (mean 3.5 days) and did not become afebrile until day 8; only 25% of these animals had a fever $\geq$ 105 F. Further, animals given 1 FD_{50} IP had low grade fevers of short duration with none > 105 F.

When sera from all the above animals were tested for phase II complement fixing (CF) antibody, reading a 3+ reaction at a serum dilution ≧ 1:8 as a positive, the titer was calculated to be $10^{5.1}$ mean CF doses per ml (CF_{50}) 14 days after inoculation and $10^{6.8}$ CF_{50} 28 days after inoculation.

Guinea pigs infected with the M44 strain converted serologically and rapidly developed high but brief febrile reactions when challenged with approximately 1 median lethal dose of the EP 88 strain. These animals appeared normal and continued to gain weight, while control animals without detectable Q fever antibody were listless and lost weight. Therefore, the reaction to phase II challenge of guinea pigs with circulating antibody is indicative of a pyrogenic reaction rather than an infection.

The antibiotic sensitivity of the M44 strain is comparable with the AD strain in that dosages of tetracycline and oxytetracycline equivalent to human doses of 2 g/day shorten the febrile response and negate the weight loss associated with infection induced by 10^4 FD_{50} per guinea pig. While 5 times this dose was no more effective, 1/5 of the dose was less effective. Chloramphenicol was not as effective as tetracycline or oxytetracycline.

Summary:

A master seed lot and a vaccine lot of the M44 strain of C. burneti have been produced; determination of the strain parameters has been initiated.

Publications:

None.

LITERATURE CITED

1. Genig, V. A. 1960. Attenuated variant "M" of Rickettsia burneti as possible live vaccine against Q fever. Vestn. Akad. Med. No. 2:46-57.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Warfare (U)**
Task No. 1B662706A096 02:	**Prevention and Treatment of Biological Warfare Casualties**
Work Unit No. 096 02 402:	**Comparative Studies of Various Routes of Immunization with Arbovirus Vaccines**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Ralph W. Kuehne, B. S.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

170

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OLO836	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	XX YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	402
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Comparative studies of various routes of immunization with arbovirus vaccines

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 08	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	10
FISCAL YEAR CURRENT 71	1	5

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign intelligence considered.

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Encephalitis, equine (EEE, VEE, WEE); (U) Arboviruses; (U) Vaccines; (U) Immunization

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Evaluate routes of and dosages for immunization of susceptible hosts with arbovirus vaccines.

24 (U) Laboratory animals will be immunized by varying routes and dosages and challenged at appropriate times subsequently.

25 (U) 69 07 - 69 06 - No work was accomplished during this report period due to movement of the laboratories to new facilities.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Warfare (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 402: Comparative Studies of Various Routes of Immunization with Arbovirus Vaccines

Description:

Evaluate routes of dosages for immunization of susceptible hosts with arbovirus vaccines.

Progress and Summary:

No work was accomplished during this report period due to movement of the laboratories to new facilities.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 403:	Cross-Immunity Within the A Group of Arboviruses
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Francis E. Cole, Jr., Ph.D. Helen T. Hargett
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

174

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
	DA OL0837	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	403
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Cross-immunity within the A group of arboviruses

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	10
FISCAL YEAR CURRENT 71	1	30

19. RESPONSIBLE DOD ORGANIZATION
NAME:* USA Medical Research Institute of Infectious Diseases
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20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hargett, H. T. DA
NAME:

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede each with Security Classification Code)
(U) Vaccines; (U) Arboviruses; (U) Immunization; (U) Encephalitis, equine (VEE, EEE, WEE)

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Evaluate experimental attenuated and inactivated group A arbovirus vaccines for their ability to induce protection against other members of the group.

24 (U) Adult hamsters are inoculated with group A arbovirus vaccines in appropriate combinations and sequences. Response is determined by challenge with virulent strains and by serological techniques.

25 (U) 69 07 - 70 06 - No studies were possible during this report period due to delays in the useful occupancy of the new USAMRIID laboratories; research will resume when facilities become available.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 403: Cross Immunity Within the A Group of Arboviruses

Description:

Evaluate experimental attenuated and inactivated group A arbovirus vaccines for their ability to induce protection against other members of the group.

Progress and Summary:

No studies were conducted during this report period due to delays in the useful occupancy of the new USAMRIID laboratories; research will resume upon acquisition of facilities.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 407:	Development of Inactivated Group A Arbovirus Vaccines
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Francis E. Cole, Jr., Ph.D. Helen T. Hargett
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0L0841	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	407
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)

(U) Development of inactivated group A arbovirus vaccines

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 06	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: NA d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING	70	1	40
FISCAL YEAR CURRENT	70	1	40

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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TELEPHONE: 301 663-4111 Ext 7241

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Hargett, H. T. DA

NAME:

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Arboviruses; (U) Encephalitis, equine (WEE, EEE); (U) Semliki Forest virus; (U) Cell culture; (U) Immunization; (U) Antibody; (U) Vaccines; (U) Roller culture

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Produce inactivated group A arbovirus vaccines from selected strains; investigate their capacity to induce serologic response and/or resistance to challenge in test animals.

24 (U) Arboviruses are propagated in primary cell cultures and inactivated with formalin. Products are tested for safety and potency in animals. Efficacy is determined by subsequent challenge.

25 (U) 69 07 - 70 06 - No studies were possible during this report period due to delays in the useful occupancy of the new USAMRIID laboratories; research will resume when facilities become available.

Publications: Appl. Microbiol. 17:927-928, 1969.
Am. J. Trop. Med. Hyg. 19:119-122, 123-126, 1970.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 407: Development of Inactivated Group A Arbovirus Vaccines

Description:

Produce inactivated group A arbovirus vaccines from selected strains of viruses propagated in cell culture; investigate the capacity of these vaccines to induce serological response and/or resistance to challenge in test animals.

Progress and Summary:

No investigations were undertaken during this report period due to delays in the useful occupancy of the new USAMRIID Laboratories; research will resume upon acquisition of facilities.

Publications:

1. Cole, F. E., Jr. and R. W. McKinney. 1969. Use of hamsters for potency assay of eastern and western equine encephalitis vaccines. Appl. Microbiol. 17:927-928.

2. Maire, L. F., III, R. W. McKinney, and F. E. Cole, Jr. 1970. An inactivated eastern equine encephalomyelitis vaccine propagaged in chick-embryo cell culture. I. Production and testing. Amer. J. Trop. Med. 19:119-122.

3. Bartelloni, P. J., R. W. McKinney, T. P. Duffy, and F. E. Cole, Jr. 1970. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture. II. Clinical and serological responses in man. Amer. J. Trop. Med. 19:123-126.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 02:	**Prevention and Treatment of Biological Warfare Casualties**
Work Unit No. 096 02 408:	**Role of Antibody in the Clinical Manifestations of Venezuelan Equine Encephalomyelitis**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Medical and Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Peter J. Bartelloni, Lt Colonel, MC Robert W. McKinney, Lt Colonel, MSC Francis E. Cole, Jr., Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OL0811	2. DATE OF SUMMARY* 70 07 01	REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA-CONTRACTOR ACCESS [X] YES [ ] NO

9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	408
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Role of antibody in the clinical manifestations of Venezuelan equine encephalitis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 02	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: NA d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING 70	1	5
CURRENT 71	1	10

19. RESPONSIBLE DOD ORGANIZATION

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ADDRESS:* Fort Detrick, Md 21701

RESPONSIBLE INDIVIDUAL

NAME:

TELEPHONE:

20. PERFORMING ORGANIZATION

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TELEPHONE: 301 663-4111 Ext 6135

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Francisco, N. M.

NAME: Cole, F. E., Jr. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Prophylaxis; (U) Encephalitis, equine (VEE); (U) Virus diseases; (U) Immune serum

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study the role of antibody as it relates to the clinical manifestations of Venezuelan equine encephalitis (VEE) virus infection, and the usefulness of immune serum in prophylaxis and treatment of this infection.

24 (U) Animals are inoculated with either attenuated or virulent VEE virus. The efficacy of antiserum in preventing undesirable reactions to these viruses is evaluated. The resulting immune response and its dependency on the relationship of the quantity of antiserum given to time of its administration are investigated.

25 (U) 69 07 - 70 06 - VEE-immune globulin, human (VEE-IG) was inoculated into volunteers infected at various times with live attenuated VEE virus (vaccine strain). Neutralizing antibodies were not detectable on day 28 in the 6 men given VEE-IG alone. They were detected in the remaining 6 men who were given VEE vaccine alone. In the 18 remaining volunteers, given VEE vaccine and VEE-IG at different intervals with respect to each other, there was no difference from results observed in VEE vaccinated men. Systemic and local reactions were mild to moderate. VEE-IG did not appear to ameliorate the effect of live VEE vaccine in men.

*/ available to contractors upon originator's approval

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 408: Role of Antibody in the Clinical Manifestations of Venezuelan Equine Encephalomyelitis

Description:

Study the role of antibody as it relates to the clinical manifestations of Venezuelan equine encephalomyelitis (VEE) virus infection, and the usefulness of immune serum in prophylaxis and treatment of this infection.

Progress:

Venezuelan equine encephalomyelitis Immune Globulin, Human (VEE-IG) (Project No. FY 69-5) was studied in volunteers. VEE-IG, Lot 0750D030A1, was prepared by Hyland Laboratories, Los Angeles, Calif. The VEE-IG was produced and tested in conformance with Public Health Service specifications for Immune Serum Globulin (Human) U.S.P. It was prepared from the plasma of donors immunized with attenuated VEE vaccine. This product had a serum neutralization index (SNI) of approximately 1.8 logs.

The purpose of this study in humans was to document and define the appearance and persistence of circulating antibody following the administration of VEE-IG and to determine the effect of this globulin on infections with attenuated VEE vaccine, NDBR 102-4 (National Drug Co., Philadelphia, Pa.).

Thirty volunteers were divided into 3 groups designated, I, II and III and administered VEE-IG and/or VEE vaccine as shown in Table I. The sera obtained on the bleeding dates shown were tested for VEE hemagglutination inhibiting (HI) and neutralizing antibodies.

All subjects in Group I administered VEE-IG alone failed to exhibit HI antibody (i.e., <1:10) and members of this group similarly failed to attain significant levels of VEE neutralizing antibody by day 28. The import of these results is readily reflected in the results obtained with Groups II-A, II-B and III where all individuals administered attenuated VEE vaccine developed an active immunizing infection; this occurred whether VEE vaccine was given before or after VEE-IG; SNI of members of these 3 groups ranged from 1.1 to 3.2 logs. These titers are comparable to those obtained from the vaccine controls (II-C) which ranged from 1.4 to 3.4 logs.

TABLE I. EXPERIMENTAL SCHEME FOR EVALUATION OF VEE-IG AND SNI DAY 28 POSTVACCINE ADMINISTRATION

GROUPS (No. of men)	PROCEDURE		RECIPROCAL HI TITER Day 28	MEAN LOG_{10} SNI
	VEE Vaccine[a]	VEE-IG[b]		
I (6)	-	Day 0	<10	0.4
II-A (6)	Day 0	+4 hr	10-20	2.5
B (6)	Day 0	+24 hr	10-320	2.4
C (6)	Day 0	-	10-40	2.3
III (6)	Day +15	Day 0	<10-40	2.3

a. Standard dose: $10^{3.7}$ guinea pig intraperitoneal immunizing doses.
b. 10 ml intramuscularly in 2 divided doses.

It is apparent that this immune globulin administered as a 10 ml-dose was not effective in preventing infection with the vaccine strain of VEE virus.

In addition, from the limited data available it does not appear that the immune globulin administered as described affected the reaction rate to attenuated VEE vaccine.

No further studies of VEE-IG in man are contemplated in the near future.

Summary:

VEE-IG was studied in volunteers. VEE antibody could not be detected in the sera of volunteers following the intramuscular administration of 10 ml of this preparation. The immune globulin did not prevent infection with live VEE vaccine when administered either 4 or 24 hr after or 15 days prior to vaccination.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 409:	Host-parasite Relationships in Virus Immunization
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Animal Assessment
Period Covered by Report:	1 July 1969 through 30 June 1970
Professional Authors:	Richard O. Spertzel, Major, VC (I) J. Brent Rollins, Captain, VC (I, II) Robert H. Fiser, Jr., Captain, MC (II) William R. Beisel, M.D. (I) George A. Burghen, M.D. (I)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0861	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	409
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Host-parasite relationships in virus immunization

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 07	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	20
FISCAL YEAR CURRENT 71	1	20

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: 301 663-4111 Ext 7244
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Rollins, J. B.
NAME: DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Virus disease; (U) Vaccines; (U) Immunization; (U) Yellow fever; (U) Encephalitis (VEE); (U) Infectious canine hepatitis

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate various interactions between animal hosts and virus vaccines.

24 (U) Various virus vaccines are given to laboratory animals or man; responses are measured.

25 (U) 69 07 - 70 06 - Dogs infected with virulent infectious canine hepatitis (ICH) and fed a normal diet showed typical clinical laboratory and histopathological results as well as large changes in alpha-2 glycoglobulin. Control animals and animals receiving attenuated ICH vaccine showed no such changes. Sequential comparison of data shows that alpha-2 glycoglobulin alterations occur very early, sometimes preceding fever. Six dogs, fed a high fat diet and given virulent ICH virus, showed typical changes as described above, but had a more rapid death rate than the dogs on a normal diet. Lipid studies of frozen plasma samples to elucidate possible mechanisms are pending.

A combined yellow fever and Venezuelan equine encephalitis vaccine previously reported in this work unit has been transferred to work unit 096 02 004.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 409: Host-parasite Relationships in Virus Immunization

Description:

Investigate various interactions between animal hosts and virus vaccines.

Progress, Part I:

The present study was designed to compare serial serum protein and glycoprotein responses in immune and nonimmune beagle dogs infected with either virulent infectious canine hepatitis (ICH) or attenuated ICH (vaccine strain) virus.

The 44 dogs used were 4-6 mon old, purebred beagles maintained in isolation since weaning and fed a commercial puppy chow. They were grouped according to the virus received and their pre-exposure immune status into one of the following 5 categories: (1) 5 nonimmune dogs were given 1 standard immunizing dose of attenuated tissue culture-origin vaccine strain ICH virus subcutaneously (SC); (2) 12 nonimmune dogs were given 1 ml Cornell strain virulent ICH intraperitoneally (IP); (3) 6 immune dogs were given the attenuated vaccine strain ICH virus; (4) 12 immune dogs were given virulent ICH virus; (5) 6 dogs used as controls were not given an injection.

To determine the immune status before and subsequent to inoculation, ICH serum neutralization tests were done on each dog on days 0 and 28.

After infection with either the virulent or the vaccine strain of virus, daily clinical examination and temperature recordings were accomplished on each dog. Clinical laboratory parameters examined include total white blood cell (WBC) count, differential leukocyte count, packed cell volume, hemoglobin, serum glutamic oxalacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT). Serum and glycoprotein electrophoresis was also done.

Most dogs were bled on days -5, -3, 0 (immediately prior to inoculation with live virus), days 1-10 inclusive and day 28, but because individual dogs were studied over a 2-yr period, minor differences occurred in the bleeding schedule in each group. In no case was an average group value represented on any day by less than 3 dogs from that group.

In addition to the 5 groups mentioned above, plastic liver windows for obtaining serial liver biopsies were implanted surgically in 3 other nonimmune dogs 5 days prior to inoculation. One dog was challenged with virulent ICH virus, one was injected with attenuated ICH vaccine, and one served as a con-

trol. Biopsies, approximately 1 gm in weight, were taken from the liver edge under direct visualization on days 0, 2, 4, and 6. All liver biopsies from each dog studied showed histologic changes attributable to the presence of the plastic windows. These changes were characterized by centrilobular degeneration or necrosis of hepatocytes with suppurative reaction in the subcapsular locations proximal to the liver window.

The serial liver biopsies taken from the nonimmune dog infected with virulent virus showed widespread intranuclear inclusions and disseminated focal areas of hepatocellular necrosis typical of ICH infection. These changes were evident by day 4 and maximal by day 6. The ICH-related, pathologic changes in this dog were concurrent with the changes attributed to the presence of the plastic window, while the control and vaccine strain-inoculated dogs from which biopsies were taken showed only the liver window-induced changes.

Highly significant changes with $p < 0.001$ were present only in the nonimmune dogs infected with virulent ICH virus. The changes were characterized by a marked progressive decrease in serum albumin, a marked, but transient, early rise in α_2-globulin and late rise in γ-globulin.

Each fraction of the glycoproteins showed larger percentage changes in response to infection than the corresponding serum protein fractions. There was a significant marked fall in glycoalbumin and α_1-glycoglobulin, and a spiking of α_2-glycoglobulin to virtually double baseline values. β-glycoglobulin fell; there was a small late rise of γ-glycoglobulin. All alterations between days 3 and 5 were statistically significant with $p < 0.001$. Abnormal α-globulin and α-glycoglobulin values from all infected dogs had returned to normal or near normal by day 28.

Electrophoretic patterns for nonimmune dogs given vaccine strain of virus produced slight changes similar to the infected group. This was particularly evidenced by the slight, though not significant, increase in α_2-glycoglobulin and the fall in β-glycoglobulin.

Clinically, all nonimmune dogs infected with virulent virus responded with fever, lethargy, vomiting, diarrhea, petechiae of mucous membranes and thin-skinned areas, and dependent edema. Sequentually, the temperature increased between days 2 and 4, and was followed by leukopenia; SGOT and SGPT values began to rise about day 5 or 6. Death occurred in 42% of this group between days 5 and 8, while dogs that recovered were clinically normal within 2 weeks after onset of symptoms. Typical hepatic necrosis and intranuclear inclusions were found when tissues of infected dogs that died were examined histologically.

α_2-glycoprotein changes begin early in this sequence, frequently being increased on day 2 and reaching a peak on day 4 or 5. This timing closely paralleled the temperature rise. Increase of α_2-glycoglobulin actually preceded temperature rise in 2 nonimmune dogs of the group infected with virulent virus.

No clinically ill dogs were found in any of the other groups, as judged by the above-mentioned parameters.

Similar changes have been attributed to other acute infections and various noninfectious inflammatory conditions, indicating a nonspecific reaction. One theory proposed for these changes is release of glycoprotein from damaged tissue. Evidence that cellular damage or destruction is not the mechanism of glycoprotein alteration is that pattern changes in experimental ICH have occurred prior to hepatocellular necrosis. This can be said because serum enzymes indicative of necrosis have not risen, and there is no histologic evidence of hepatocellular damage until day 4. In contrast, glycoprotein pattern changes are evident by day 2.

Lack of highly significant electrophoretic change, in either nonimmune dogs that received an attenuated virus, or in immune dogs that received virulent virus, indicates that entry or presence of an infectious microorganism does not create abnormal patterns. Rather, abnormal patterns are the result of host response associated with occurrence of disease, secondary to the detrimental effects of the virus.

Summary, Part I:

Nonimmune dogs infected with virulent ICH virus responded with fever, leukopenia, α_2-glycoglobulin increase, decreased serum albumin and death in 42% of the cases, while nonimmune dogs given attenuated vaccine and immune dogs given virulent virus showed minimal or no response.

Progress, Part II:

Half of 12 nonimmune beagle dogs were fed military stress diet (MSD) containing high fat content for 6 weeks prior to challenge, while the other half received regular Purina diet. Weight gain, hematology, lipoproteins, cholesterol, free fatty acids, triglycerides, total protein, and serum and glycoprotein electrophoresis were monitored during this time and subsequent to challenge with virulent ICH virus.

Weight gain in the MSD group was slightly lower during the 6-week period, but the MSD group lost less weight after challenge than the Purina-fed group. The MSD group looked clinically fatter, even though their rate of gain as a group was slightly lower than the Purina-fed dogs.

While all dogs suffered fever, leukopenia, lethargy, and a variety of other signs, death occurred more rapidly in the MSD-fed dogs. Interpretation of this finding is under discussion and may be answered when all the above-mentioned tests are completed.

Lipoprotein electrophoresis has been completed and results show that the MSD group has much more densely stained lipoprotein electrophoretic strips.

Summary, Part II:

Beagle dogs fed a high fat diet suffered a more rapid death rate in response to infection than dogs that received a normal diet. Interpretation of this finding may be elucidated by lipid studies on the frozen plasma from the 2 groups of dogs.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 410:	Pathophysiology and Treatment of Yellow Fever
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Animal Assessment and Pathology
Period Covered by Report:	1 July 1969 through 30 June 1970
Professional Authors:	Frank E. Chapple, III, Captain, VC Richard O. Spertzel, Major, VC Jerry D. Weil, Captain, VC Douglas W. Mason, Captain, VC Ulysses McElyea, Jr., Captain, VC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OL0877	2. DATE OF SUMMARY* 70 07 01	REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA - CONTRACTOR ACCESS XX YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	410
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Pathophysiology and treatment of yellow fever

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 12	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	3	30
FISCAL YEAR CURRENT 71	3	30

19. RESPONSIBLE DOD ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Spertzel, R. O.
NAME: Mason, D. W. DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Yellow fever; (U) Therapy; (U) Cross-circulation

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study cross-circulation as a mode of therapy for yellow fever.

24 (U) Inoculate rhesus monkeys with Asibi strain yellow fever virus. Study changes in blood and tissues during the course of the disease. Study cross-circulation as a mode of therapy.

24 (U) 69 07 - 70 06 - Cross-circulation of yellow fever-infected monkeys has been suspended. Many of the technical problems encountered have been overcome and the number of deaths that previously occurred with normal monkeys following cross-circulation has been greatly reduced.

*Available to contractors upon originator's approval.

DD FORM 1498 1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 410: Pathophysiology and Treatment of Yellow Fever

Description:

Study cross-circulation as a mode of therapy for yellow fever in the rhesus monkey.

Progress:

Due to the move to the Phase I facility, studies using virulent yellow fever virus have been suspended. During the year, however, several cross-circulation trials have been conducted, using normal, noninfected animals. Through these trials, it has been established that the use of Dicumerol will prevent the formation of clots during cross-circulation.

Summary:

Although cross-circulation of yellow fever-infected monkeys has been suspended, many of the technical problems encountered have been overcome. The number of deaths that previously occurred with normal monkeys following cross-circulation have been greatly reduced.

Presentations:

1. Chapple, F. E., III, J. M. Crosbie, B. E. Reisberg, and R. O. Spertzel. Surgical technique for cross-circulation of rhesus monkeys. Presented at American Association for Laboratory Animal Science, Dallas, Texas. 13-17 October 1969.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 02:	Prevention and Treatment of Biological Warfare Casualties
Work Unit No. 096 02 800:	Development of a Polyvalent S. aureus Toxoid
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Pathology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Joseph F. Metzger, Colonel, MC Anna D. Johnson
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OL0895	2. DATE OF SUMMARY* 70 07 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 70 05 20	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA-CONTRACTOR ACCESS [X] YES [ ] NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	02	800
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Development of a polyvalent S. aureus toxoid

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 04	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	1	15
	CURRENT 71	1	15

19. RESPONSIBLE DOD ORGANIZATION
NAME:* USA Medical Research Institute of Infectious Diseases
ADDRESS:* Fort Detrick, Md 21701

RESPONSIBLE INDIVIDUAL
NAME: Crozier, D.
TELEPHONE: 301 663-4111 Ext 5233

20. PERFORMING ORGANIZATION
NAME:* Pathology Division
USA Medical Research Institute of Infectious Diseases
ADDRESS:* Fort Detrick, Md 21701

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Metzger, J. F.
TELEPHONE: 301 663-4111 Ext 2134
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Johnson, A. D. DA
NAME:

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Enterotoxin; (U) Staphylococcus; (U) Toxoid

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop a polyvalent toxoid which would include Staphylococcus aureus enterotoxins A, B, C, and D and other exoproteins.

24 (U) Initial studies would include preparation of apilot bach of formalinized broth filtrate utilizing organism 10-275 (potent producer of alpha hemolysin and B toxin). Determine cultural and toxoiding conditions.

25 (U) 70 04 - 70 07 - A 10-L fermenter culture of strain 10-275 S. aureus was grown in a completely dialyzable media. The resultant crude broth contained 150 micrograms of B toxin and 256 units of alpha hemolysin. Toxoiding safety tests were satisfactory at 21 and 28 days. On the 28th day, a 10-L batch was dialyzed for 1 week against phosphate buffered saline with 1:10,000 merthiolate. Filtration through a millipore filter (HA) was accomplished and the final product was bottled aseptically in 100 ml vaccine bottles Further tests will be conducted.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 02: Prevention and Treatment of Biological Warfare Casualties

Work Unit No. 096 02 800: Development of a Polyvalent S. aureus Toxoid

Description:

Develop a polyvalent toxoid which would include Staphylococcus aureus enterotoxins A, B, C, and D and other exoproteins.

Progress:

Enterotoxicity due to exoproteins of S. aureus is common in institutional environments and in the military situation. Four enterotoxins designated A, B, C, and D have been described. Dack, et al.[1] and Dolman[2] utilized toxic filtrates administered per os for immunization of animals and volunteers. Davison and his co-workers[3] produced active immunity in monkeys utilizing subcutaneous injections of formalinized filtrate. Bergdoll, et al.[4] reported that the different enterotoxins did not cross react or cross protect. The development of a polyvalent (A, B, C, and D[5]) enterotoxoid would make possible an immunization which might offer protection against all enterotoxins of S. aureus thus far identified. In addition, it is possible that antibodies which might develop to the other exoproteins and polysacchrides present in crude broth filtrates could offer protection against local and systemic staphylococcal infection.

Initial studies concerned the cultivation of S. aureus (10-275) in casaminoacids supplemented with a dialysate of yeast extract. Cultivation for 18 hr in a 10-L fermenter under 5% CO_2 and 95% air resulted in production of 160 μg of B toxin (as determined by radial diffusion technique) and 256 units of α-hemolysin. Other exoproteins and polysaccharides were not quantitated.

The organisms were removed by centrifugation at 15,000 rpm in a continuous flow head. Formalin was added and aliquots were removed after 1, 3, and 4 weeks. All formalinized filtrates were dialyzed against phosphate buffered saline containing 1:10,000 merthiolate for a minimum of 72 hr. After dialysis, the filtrates were filtered through a millipore filter and dispensed aseptically in 100-ml vaccine bottles.

Cooperating investigators include Dr. V. McGann, Bacteriology Division, who is involved with quantitation of toxin and antibody studies on animals, and members of the Animal Assessment Division who perform safety tests and animal immunization.

Summary:

A 10-L fermenter culture of 10-275 was grown in a completely dialyzable media. The resultant crude broth contained 160 μg of B toxin and 265 units of α-hemolysin. Toxoiding safety tests were satisfactory at 21 and 28 days. On the 28th day, a 10-L batch was dialyzed for one week against phosphate buffered saline with 1:10,000 merthiolate. Filtration through a millipore filter (HA) was accomplished and the final product was bottled aseptically in 100-ml vaccine bottles.

Publications:

None.

LITERATURE CITED

1. Dack, G.M., E.O. Jordan, and O.C. Woolpert. 1931. Attempts to immunize human volunteers with staphylococcus filtrates that are toxic to man when swallowed. J. Prev. Med. 5:151-159.

2. Dolman, C.E. 1943. Bacterial food poisoning. Canad. J. Pub. Health 34:205-235.

3. Davison, E., G.M. Dack, and W.E. Cary. 1938. Attempts to assay the enterotoxic substance produced by staphylococci by parenteral injection of monkeys and kittens. J. Infect. Dis. 62:219-223.

4. Bergdoll, M.S., M.J. Surgalla, and G.M. Dack. 1959. Staphylococcal enterotoxin. Identification of a specific precipitating antibody with enterotoxin-neutralizing property. J. Immun. 83:334-338.

5. Casman, E.P., M.S. Bergdoll, and J. Robinson. 1963. Designation of staphylococcal enterotoxins. J. Bact. 85:715-716.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	**Medical Defense Aspects of Biological Agents (U)**
Task No. 1B662706A096 03:	**Laboratory Identification of Biological Agents**
Work Unit No. 096 03 003:	**Diagnosis of Viral Infections with Homologous Bone Marrow Cultures**
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Francis E. Cole, Jr., Ph.D.
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

200

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0850	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	XX YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	003
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9); 1412a(2)			

11. TITLE (Precede with Security Classification Code)*
(U) Diagnosis of viral infections with homologous bone marrow cultures

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 03	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	0	0
	CURRENT 71	1	5

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Bone marrow; (U) Encephalitis, equine (VEE); (U) Arbovirus infection; (U) Tissue culture

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop a diagnostic system in which bone marrow serves as both a clinical specimen and a host system for in vitro virus propagation; determine the duration of bone marrow infection in animals inoculated with the attenuated strain of VEE virus; define the correlation between persistence of infection and maintenance of antibody level.

24 (U) Canine hosts are inoculated with attenuated or Trinidad strain VEE virus. At various intervals postinoculation the animals are bled for viremia and hemagglutination-inhibition antibody assays, and samples of marrow are aspirated from the ilium for use in cultural procedures. After incubation for periods up to 10 days at 37 C, virus content of culture fluids is assayed by the mouse protection test.

25 (U) 69 07 - 70 06 - Due to shift of personnel to work of higher priority, no studies were conducted. Work will resume when sufficient personnel are available.

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 003: Diagnosis of Viral Infections with Homologous Bone Marrow Cultures

Description:

Develop a diagnostic system in which bone marrow serves as both a clinical specimen and a host system for in vitro virus propagation; determine the duration of bone marrow infection in animals inoculated with attenuated Venezuelan equine encephalomyelitis virus; define the correlation between persistence of infection and maintenance of antibody level.

Progress and Summary:

Due to shift of personnel to work of higher priority, no studies were conducted. Work will resume when sufficient personnel are available.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 006:	Early Immune Response In Infectious Diseases and Toxemia
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Bacteriology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Joseph Kaplan, Captain, MD
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

204

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OL0870	2. DATE OF SUMMARY* 70 07 01		REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA - CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	006
b. [illegible]				
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Early immune response in infectious disease and toxemia

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	7
FISCAL YEAR CURRENT 71	1	7

19. RESPONSIBLE DOD ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Kaplan, J.
TELEPHONE: 301 663-4111 Ext 7341
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered.

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Immunology; (U) Antibody formation; (U) Chemotaxis; (U) Hemagglutination; (U) Complement; (U) Macrophage; (U) Enterotoxin; (U) Staphylococcus

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop and employ sensitive methods for detection of circulating antigen or immunoglobulin early in the course of infectious disease or toxemia.

24 (U) Sensitivity of various assay techniques for detection of immunoglobulin, including chemotaxis, augmented hemagglutination, and macrophage migration will be determined. These will be applied to detection of circulating antibody and antibody forming cells early in the course of infection in animals and humans.

25 (U) 69 07 - 70 06 - The findings that staphylococcal enterotoxin B (SEB) inhibits the migration of peritoneal exudate cells from guinea pigs have been confirmed. Studies reported here indicate that the mechanism of this migration inhibition effect is due in part to SEB stimulation of lymphocytes to produce migration inhibition factor. It was also found that type specific antitoxin blocked the migration inhibition effect of SEB. This finding implied that the system could be made specific for detection of SEB. Dialyzed SEB toxoid preparations varied in their effect on migration; this variability may have been related to the presence of unmodified toxin. Should this hypothesis be confirmed, the technique offers the first in vitro assay for unmodified toxin in toxoided preparations.

Studies on the mechanism of action of those factors involved in migration inhibition are continuing.

Publication: Clin. Res. 18:426, 1970 (abstract).

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 006: Early Immune Response in Infectious Diseases and Toxemia

Description:

Develop and employ sensitive methods for detection of circulating antigen or immunoglobulin early in the course of infectious disease or Toxemia.

Progress:

It has been shown that sensitized lymphocytes from guinea pigs with delayed hypersensitivity can be cultured in the presence of specific antigen and will release a factor which inhibits migration of peritoneal exudate cells (a mixture of lymphocytes and macrophages) from capillary tubes.[1,2] This production by lymphocytes of a migration inhibition factor (MIF) is of potential value in detecting specific antigens. It should be possible in an animal with delayed hypersensitivity to a given antigen, to demonstrate the presence of that antigen in an unknown specimen by testing for specific inhibition of migration of the hypersensitized animal's peritoneal exudate cells.

Studies were begun using staphylococcal enterotoxin B (SEB) as the model antigen. Craig and Irvin[3] showed that SEB inhibited the migration of peritoneal exudate cells from approximately 80% of normal adult guinea pigs. At low doses, SEB inhibited the mixed population of peritoneal exudate cells, but did not inhibit the migration of purified macrophages. This suggested that inhibition by SEB was lymphocyte-dependent and that SEB stimulated lymphocyte production of MIF. These findings imply the possibility that most normal adult guinea pigs become sensitized to SEB by natural exposure.

Studies were designed to determine whether SEB induced MIF. Because SEB inhibited the migration of normal guinea pig peritoneal exudate cells, the usual method for demonstrating MIF production could not be employed. However, since purified macrophages were inhibited by MIF but not by SEB, they could be used instead of the exudate cells to detect MIF in supernatants from SEB-stimulated lymphocyte cultures. In order to demonstrate the purity of preparations, macrophages from guinea pigs sensitized to bovine γ-globulin (BGG) were used. In functionally pure macrophage preparations, i.e. absence of MIF-producing lymphocytes, BGG would not inhibit migration.

Guinea pigs were sensitized to BGG in complete Freund's adjuvant. Approximately 3 weeks later, mineral oil-induced peritoneal exudate cells were obtained, washed and suspended in culture media consisting of Eagle's Minimal Essential Medium (MEM) containing 15% normal guinea pig serum. This suspension was then drawn into capillary tubes and centrifuged. The capillary tubes were cut at the cell-fluid interface and placed in plastic culture dishes containing culture media with and without specific antigen. Purified macrophages were obtained by serial plating of peritoneal exudate cells in plastic dishes and retrieving those cells adhering to the plastic surface. With this procedure, >95% of recovered cells were macrophages. After 20 hr incubation at 37 C, migration patterns for both peritoneal exudate cells and purified macrophages were projected and traced, and the areas of migration determined by planimetry. Lymphocytes were obtained from minced axillary, inguinal and popliteal nodes. They were cultured at 37 C for 24 hr with and without antigen and the culture supernatants examined for MIF.

As shown in Table I, SEB does induce the production of MIF. It was apparent that peritoneal exudate cells were inhibited by both antigens but

TABLE I. EFFECT OF SEB AND BGG (50 μg/ml) ON MIGRATION OF PERITONEAL CELLS AND PURIFIED MACROPHAGES FROM GUINEA PIGS WITH DELAYED HYPERSENSITIVITY TO BGG (* = $p < 0.05$, ** = $p < 0.01$)

	MIGRATION, % OF CONTROL[a]					
	Exudate Cells		Purified Macrophages			
					Supernatant[b]	
EXPERIMENT NO.	BGG	SEB	BGG	SEB	BGG	SEB
1	56*	47*	83	88	78*	55**
2	0**	0**	78	160*	37*	27*

a. 100% control = the average migration of peritoneal exudate cells in media without antigen, or of purified macrophages in supernatants from lymphocytes cultured without antigen. Each value represents the average migration from at least 4 tubes.

b. Supernatants from BGG-sensitized lymphocytes cultured for 24 hr with either 50 μg/ml of BGG or SEB.

that purified macrophages were inhibited only with supernatants from lymphocytes cultured in the presence of antigen. Results of the second experiment where it is seen that there was enhancement of migration of the purified macrophages by SEB are not understood; however, it is evident that inhibition by SEB supernatant was significant, $p < 0.05$. Further studies are necessary to determine whether MIF induction by SEB is due to a naturally-acquired sensitization or a nonspecific stimulating effect on unsensitized lymphocytes.

Migration inhibition should be a sensitive method for detecting antigens. A unique problem associated with detection of SEB by this system is the apparent nonspecific inhibition of nonsensitized guinea pig cells. An approach to providing specificity would be to demonstrate an effect of antitoxin on SEB migration inhibition.

Another series of experiments was designed to determine the effect of antibody to SEB upon SEB migration inhibition. Results are shown in Table II. These results indicate that guinea pig antibody to SEB blocked SEB migration inhibition. This blocking effect appears to be inversely proportional to the ratio of toxin to antitoxin. These data indicate that this in vitro system can be made specific for SEB detection.

In an attempt to investigate the mechanism involved in SEB stimulation of lymphocyte production of MIF, it was postulated that the toxic moiety of SEB was responsible. Therefore, the effects of SEB and SEB-toxoid (Lot 87285) on migration of guinea pig peritoneal exudate cells were studied.

A sample of Lot 87285 toxoid was dialyzed at 4 C for 72 hr against MEM to remove free formalin and thimerasol. The effect of this dialyzed material on cell migration was evaluated in the manner previously described. At concentrations of 25 μg/ml, the dialyzed toxoid did not inhibit migration, whereas unmodified toxin did. To confirm this finding, the study was repeated. This dialyzed sample inhibited migration. Serological studies with both dialyzed samples demonstrated that the first preparation reacted in the same manner as untreated toxoid, whereas the second sample showed evidence of 2 antigenic components. If the second component was residual toxin and was responsible for migration inhibition, this technique would offer for the first time an in vitro assay for detecting residual toxicity.

Studies are in progress to delineate further the mechanism involved in lymphocyte production of MIF and to adapt this in vitro assay system to the rapid detection of bacterial antigens.

TABLE II. EFFECT OF SEB ON MIGRATION OF NORMAL GUINEA PIG PERITONEAL EXUDATE CELLS IN MEM MEDIA CONTAINING EITHER 15% NORMAL OR 15% GUINEA PIG ANTI-SEB SERUM (* = $p < 0.05$, ** = $p < 0.01$)[a/]

SEB µg/ml	EXPERIMENT NO.	MIGRATION, % OF CONTROL[b/]	
		Normal Guinea Pig Serum	Anti-SEB Guinea Pig Serum
100	1	11	23
	2	50	111**
10	1	7	42**
	2	66	120**
1.0	1	9	92**
	2	64	148**
0.1	1	11	103**
	2	65	132*
0.01	1	7	95**
	2	91	83
0.001	1	15	90**
	2	73	90
0 (Controls)	1	100	100
	2	100	100

a. Represents significantly greater migration in anti-SEB guinea pig serum than in normal guinea pig serum.

b. 100% control = the average migration of peritoneal exudate cells in media without antigen. Each value represents the average migration from at least 8 tubes.

Summary:

The findings that SEB inhibits the migration of peritoneal exudate cells from guinea pigs have been confirmed. Studies reported here indicate that the mechanism of this migration inhibition effect is due in part to SEB stimulation of lymphocytes to produce migration inhibition factor. It was also found that type specific antitoxin blocked the migration inhibition effect of SEB. This finding implies that the system could be made specific

for detection of SEB. Dialyzed SEB toxoid preparations varied in their effect on migration; this variability may have been related to the presence of unmodified toxin. Should this hypothesis be confirmed, the technique offers the first in vitro assay for unmodified toxin in toxoided preparations.

Studies on the mechanism of action of those factors involved in migration inhibition are continuing.

Presentations:

1. Irvin, W. S., and R. G. Sherry. The in vitro inhibition of guinea pig macrophage migration by staphylococcal enterotoxin B (SEB). Presented at National Meeting of American Federation for Clinical Research, Atlantic City, N. J. 2-3 May 1970.

Publications:

1. Irvin, W. S., and R. G. Sherry. 1970. The in vitro inhibition of guinea pig macrophage migration by staphylococcal enterotoxin B (SEB). Clin. Res. 18:426 (Abstract).

LITERATURE CITED

1. Bloom, B. R., and B. Bennett. 1966. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science 153:80-82.

2. David, J. R. 1966. Delayed hypersensitivity in vitro: Its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc. Nat. Acad. Sci., USA 56:72-77.

3. U. S. Army Medical Research Institute of Infectious Diseases. 1 July 1969. Annual Progress Report, FY 1969. p. 191 to 199. Fort Detrick, Maryland.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 008:	Mathematical and Computer Applications in Infectious Disease Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Robin T. Vollmer, Captain MC Darrell A. Leonhardt, PFC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OL0897	2. DATE OF SUMMARY* 70 07 01	REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 70 05 25	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N DE	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	008
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9); 1412a(2)			

11. TITLE (Precede with Security Classification Code)*
(U) Mathematical and computer applications in infectious disease research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 04	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	1	2
FISCAL YEAR CURRENT 71	1	5

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: Leonhardt, D. A.
NAME: DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Diagnosis; (U) Computers; (U) Medicine

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop techniques for utilization of computers, statistics and mathematics to process and interpret scientific data.

24 (U) Automation of data analysis currently accomplished with the Fort Detrick CDC 3150 computer will be expanded with a data acquisition system. The theories and disciplines of numerical analysis, differential equations, statistical tests of hypothesis, experimental design, information storage and retrieval, and pattern recognition are utilized to analyze and interpret data gathered by various investigators.

25 (U) 69 07 - 70 06 - Mathematical, statistical and computer techniques have been applied to data emanating from the Institute. The input has come from investigations on plasma growth hormone, renal physiology, amino acids, volunteer studies, and serum proteins.

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 008: Mathematical and Computer Applications in Infectious Disease Research

Description:

Develop techniques for utilization of computers, statistics and mathematics to process and interpret biological data derived during studies of infectious disease or toxemia.

Progress:

In the past year research has been conducted in the applications of mathematics, statistics, and computer science to various study projects of the Institute. Efforts to automate data analysis have been extended both by the use of the Fort Detrick CDC 3150 computer and by plans to procure an automated data acquisition system (DAS). This DAS will "listen to", digitize, and further process the analog signals from the amino acid analyzer and from the electrophoresis densitometer as well as recording the digital output (counts per minute) from a double channel particle counter. Furthermore, it will be expandable to interface in the future with more analog inputs such as the autoanalyzers and with additional digital inputs such as a β-particle counter. The output of the DAS will be either stored in computer compatible form for further analysis or printed out for immediate examination.

The formation and subsequent use of growth hormone radioimmunoassay standard curves to calibrate unknown samples have been automated (Work Unit 096 01 002) The algorithms involved first convert the counts per minute to per cents, from which a standard curve is derived. The curve is chosen from 3 mathematical models, each of which has been fitted to the standard data. An iterative technique is used in this fitting procedure in order to alter the parameters of the model to obtain a "best" fit. Then the model yielding the smallest sum of squared deviations from the standard data is chosen for the standard curve, and all unknown samples are automatically calibrated. The unknown growth hormone values together with a copy of the standard curve are printed on paper by the computer.

Renal physiology data analysis has also been automated (Work Unit 096 01 001). Using a set of 286 measurements from each monkey undergoing study, 22 renal physiological parameters are calculated for each 20-min period of the 11 hr period of study. A control period average and variance are calculated, and the results along with the monkey number are stored temporarily in the computer's auxiliary memory until all monkeys have been analyzed. Then a statistical program groups the data and tests for significant differences in means between groups by calculating t values (Student's t distribution). The data are analyzed both before and after correcting for individual animal differences by expressing the results as per cents of the uninfected control period averages. Finally, least squares regression coefficients are calculated for pairs of parameters felt to be correlated.

Amino acid data analysis has been partially automated with a program to collect amino acid measurements in biochemically meaningful amino acid groups (Work Unit 096 01 009). This is done separately for each time period of the study, and then a control period average and variance are calculated. Again the results are expressed either in raw form or as per cent of control period averages. Statistical t tests are done to detect significant differences in means between infected and noninfected groups.

Although the statistical hypothesis testing performed in the Institute has utilized primarily the t test, research is under way to develop the appropriate mathematical models and procedures to use factorial analysis, analysis of variance, and analysis of covariance techniques to test for significant effects of infection.

A computer data bank is being created to store the clinical, physiological, and biochemical data collected during the volunteer sandfly fever projects conducted on Ward 200. The data will be stored in the computer auxiliary memory such that they can be easily searched and brought into the computer for various kinds of analyses including statistical tests for significance and correlation analysis.

A multicompartmental analysis approach is being used for the study of lipid kinetics during infection (Work Unit 096 01 013). Initial estimates of rate constants are obtained using a graphic "peeling" technique. Final estimates will be obtained using the SAAM-25 program developed at National Institutes of Health by Berman and Weiss.[1/]

Finally, plans have been developed to apply pattern recognition techniques to the area of serum protein electrophoretic patterns in an attempt to recognize subtle changes that may occur in the serum proteins early in infection (Work Unit 096 01 009).

Summary:

Mathematical, statistical and computer techniques have been applied to data emanating from the Institute. The input has come from investigations on plasma growth hormone, renal physiology, amino acids, volunteer studies, and serum proteins.

Publications:

None.

LITERATURE CITED

1. Berman, M., and M. F. Weiss. 1966. SAAM Manual, Public Health Service Publication No. 1703, U. S. Government Printing Office, Washington, D. C.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 009:	Application of Electron Spin Resonance Spectrometry to Infectious Disease Research
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	William J. Caspary, Captain, MSC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

218 JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0898	70 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 05 28	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	009
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9); 1412a(2)			

11. TITLE (Precede with Security Classification Code)*

(U) Application of electron spin resonance spectrometry to infectious disease research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 04	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	1	2
	CURRENT 71	1	7

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign intelligence considered.

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Spectroscopy; (U) Biochemistry; (U) Electron spin resonance; (U) Spin labels

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Show usefulness of electron spin resonance spectroscopy in solving problems related to infectious diseases.

24 (U) Search for the effect of infectious agents and toxins on biochemical reactions involving free radicals. Spin label various compounds involved in fectious or immunologic processes.

25 (U) An electron spin resonance spectrometer has been acquired. This equipment adds a new capability as a method for monitoring biochemical reactions during infection or toxemia. It is being calibrated preparatory to making pilot runs.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 009: Application of Electron Spin Resonance Spectrometry to Infectious Disease Research

Description:

Show usefulness of electron spin resonance spectrocopy in solving problems related to infectious diseases.

Progress:

Free radicals are suspected intermediates in a number of biochemical reactions and have been detected in altered amounts in certain diseased tissues. If these free radicals can be detected, and studied serially in key host tissues, the particular biochemical reaction could be monitored for the effects caused by infectious and toxic agents.

In order to carry out such studies, we have recently obtained an electron spin resonance spectrometer. This machine is able to detect free radicals directly by causing transitions between spin energy levels. The signals are proportional to the concentrations of free radicals in the sample cell.

Another approach to the study of infection involves the use of spin labels. These are essentially small, minimally reactive free radicals which can be attached chemically to a biologic molecule of interest. Spin labels provide a technique similar to radioactive and fluorescent labeling that has been so successful in biological problems. They offer the advantage, however, of being able to provide information not only on the existence but also on the physical conformation of the labeled molecule.

The spectrometer has been received and is being calibrated. Pilot studies have been planned and are being initiated.

Summary:

An electron spin resonance spectrometer has been acquired. This equipment adds a new capability as a method for monitoring biochemical reactions during infection or toxemia. It is being calibrated preparatory to making pilot runs.

Publications:

None.

ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 010:	Trace Metal Changes during Infectious Disease
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Physical Sciences
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Robert S. Pekarek, Ph.D. (I-III) Peter J. Bartelloni, Lt Colonel, MC (I) Karen Bostian, B. S. (I-III) Jean B. DuBuy, Captain, MC (II) Robert W. Wannemacher, Jr., Ph.D. (III) James B. Walford, III, Captain, VC, (III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

222 JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0899	70 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 05 28	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	010
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9); 1412a(2)			

11. TITLE (Precede with Security Classification Code)*

(U) Trace metal changes during infectious disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 01	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* NA
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	10
FISCAL YEAR CURRENT 71	2	10

19. RESPONSIBLE DOD ORGANIZATION

NAME:* USA Medical Research Institute of Infectious Diseases
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NAME: Walford, III, J. B. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Trace metals; (U) Endogenous mediator; (U) Bacterial infections; (U) Viral infections; (U) Monkey models

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Assess trace metal changes during infectious illness as a possible aid in early identification of disease etiology.

24 (U) Measure serum trace metals during infectious illness of laboratory animals and man and determine mechanisms responsible for observed changes.

25 (U) Early significant decreases in serum Fe and Zn levels with a concomitant rise in serum Cu were demonstrated in humans infected with either acute bacterial or viral infections. Recent animal studies have shown that infection-induced alterations in trace metal metabolism may be initiated by an endogenous mediator which is released, in part, by neutrophils.

Rhesus monkeys are being employed as a model for studying the effects of gram + and gram - bacterial infections on trace metal metabolism.

Publications:
Am. J. Med. Sci. 258:14-25, 1969.
Appl. Microbiol. 18:482-484, 1969.
Fed. Proc. 29:297, 695, 1970 (abstracts).
Bact. Proc. 1970. p. 81.
Am. J. Clin. Nutr. 23:660 (abstract).

*Available to contractors upon originator's approval.

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 010: Trace Metal Changes during Infectious Disease

Description:

Assess trace metal changes during infectious illness as a possible aid in early identification of disease etiology.

Progress, Part I:

Recent prospective clinical studies in human volunteers have demonstrated that serum Fe, total Fe-binding capacity, and serum Zn levels fall early after exposure to either an acute bacterial (Pasteurella tularensis) (Project No. FY 68-4) or a viral (attenuated Venezuelan equine encephalitis vaccine) infection (Project No. FY 69-1), with a concomitant rise in serum Cu concentrations.[1,2] Alterations in these serum metal concentrations were significantly different from changes expected on the basis of day-to-day variability or differences among individuals. The prospective nature of these studies and the rapid development of altered serum metal metabolism, even in exposed subjects who remained asymptomatic, suggest that the changes in these metals in the prodromal period represent an early host response to the presence of invading microorganisms. In subjects who developed febrile illness, the responses became exaggerated and appeared to be related in timing and magnitude to the onset and severity of the febrile reaction. The possibility was raised that an endogenous mediating factor was released from polymorphonuclear leukocytes and served to trigger the observed changes in the metabolism of these metals. Thus, these infection-induced changes in trace metal metabolism may have significance in providing new approaches to diagnosis and to an improved understanding of metabolic responses during infection.

More recently, alterations in serum Fe, Zn, and Cu concentrations were studies prospectively in a group of volunteers infected with sandfly fever virus (the Sicilian type) (Project No. FY 70-1). Based on fever (rectal temperature > 100 F), the subjects were divided into 3 clinical response categories: typical illness, mild illness and asymptomatic. Significant decreases in serum Fe and Zn concentrations and a rise in serum Cu were observed only in those individuals who developed either mild or typical illness. Unlike the previous studies, where moderate alterations occurred in the prodromal period, alterations in the serum metals during sandfly fever infection occurred on the day of onset of fever. However the magnitude of these alterations appeared to be related to the degree of the febrile response.

Besides examining changes in the serum concentrations of the above metals in response to sandfly fever, an attempt was made in conjunction with Mr. Joseph T. Piechocki, Division of Biochemistry, Walter Reed Army Institute of Research, to measure changes in serum Cr concentrations. However, the range of serum Cr concentrations in normal young adult males was found to be quite low during pre-exposure baseline control measurements and at the limits of technical measurability. No increase in Cr occurred during sandfly fever; because of low baseline values a possible decline in Cu values could not have been documented, even if present.

Summary, Part I:

Significant alterations in Fe, Zn, and Cu metabolism were demonstrated in volunteers exposed to either acute bacterial or viral infections. The prospective nature of these studies demonstrated that these alterations occur as an early host response to infection, with the magnitude of these changes showing a close relationship to the degree of febrile illness.

Progress, Part II:

Studies in animal models have shown increasing evidence that the release of an endogenous mediator from polymorphonuclear leukocytes (PMN) may be directly or indirectly involved in the production of hypoferremia and serum Zn reductions in the infected host.[3,4] This endogenous factor was shown to be present in the serum within 2 hours postendotoxin intoxication in the cat and in a large enough concentration to permit its Fe and Zn depressing effects to be transferred to normal or endotoxin tolerant recipient rats. This factor could be destroyed by heating the serum at 90 C for 30 min. To exclude the possibility that these changes may have been due to transfer of either a modified endotoxin or an endotoxin-protein complex, transfer studies were performed using virulent Diplococcus pneumoniae (a gram positive bacterium that does not produce endotoxin), an attenuated vaccine strain of P. tularensis, and polyinosinate-polycytidylate (Poly I: Poly C), a synthetic double stranded RNA which has been shown to induce endogenous interferon. In all cases, the transfer of millipore-filtered 2-hr serum from these infected or intoxicated donor rats significantly depressed serum Fe and Zn concentrations in respective groups of normal recipient rats. These experiments tend to support the hypothesis of the release of a common endogenous mediator of trace metal metabolism during infection and inflammation.

In order to determine if the endogenous mediator was released by PMN, peritoneal exudates were obtained from rats and a factor released from leukocytes during incubation in normal saline was isolated. When administered intraperitoneally to endotoxin tolerant rats, the leukocytic factor produced significant decreases in serum Fe and Zn which were linear to the logarithm of the doses administered. Similar results also were obtained in rabbit and monkey model systems. A leukocytic factor thus appeared to serve as an endogenous mediator of Fe and Zn depression.

Partially purified samples of the endogenous mediator were obtained from 2-hr sera of infected or intoxicated animals as well as from peritoneal leukocyte incubation fluid by either a methanol-butanol extraction method or by treatment with varying concentrations of phenol. Preliminary data indicate that the partially purified mediator from this serum and from peritoneal leukocytes is a heat labile, nondialyzable, low molecular weight protein (20,000-30,000 as determined by ultrafiltration technique), which is soluble in organic solvents.

These initial characteristics for the endogenous mediator of trace metals are similar to those cited for endogenous pyrogen. However the endogenous mediator of trace metal metabolism during infection and toxemia shows less species specificity than does the endogenous mediator of fever. Both fever and trace metal alterations were observed when different animal species received homologous preparations of the mediator. When these animals received heterologous preparations, only changes in trace metals were observed. Studies are now in progress to purify further the endogenous mediator, to characterize its mechanism of action, and to assess its possible effect on other biochemical and metabolic parameters during infection. Appearance of the endogenous mediator of trace metal metabolism in serum may be one of the earliest detectable responses of the host to invasion by infecting microorganisms.

In a cooperative study with Dr. R. L. Squibb, Rutgers University, New Brunswick, N. J., (Contract No. DA-49-193-MD-2694) the effects of Newcastle disease virus (NDV) on serum diurnal patterns of Cu, Zn, cholesterol, and carotenoid in chicks were investigated. All chicks were fed the same diet and were maintained with constant light. Comparative changes were observed in 4 wk old white Leghorn male chicks starting 48 hr prior to NDV inoculation and continuing 120 hr postinoculation. Serum samples were taken from normal and NDV chicks at 0800, 1500, 2000 and 2400 hours each day.

In controls, serum Cu had the most striking diurnal pattern with troughs at 2000 and daily changes of as much as 80%. Maximum daily variations in Zn were 50%, cholesterol 26% and carotenoids 28%. NDV involvement: (1) significantly increased serum Cu in 16 hr and continued throughout the entire period; (2) depressed Zn values starting at 12 hr; (3) depressed the cholesterol level slightly for up to 48 hr followed by a significant increase for the remainder of period; (4) depressed carotenoid values throughout the 120 hr. Data indicate NDV resulted in almost immediate and significant biochemical changes in serum components prior to any clinical manifestations of disease.

Summary, Part II:

Recent studies in animal models have shown that infection or toxin-induced depressions in serum Fe and Zn concentrations may be mediated by an endogenous mediator released, in part, from PMN. Preliminary data indicate that the mediator of altered trace metal metabolism is a heat labile,

nondialyzable, low molecular weight protein (20,000-30,000), which is soluble in organic solvents. Although similar in many respects to endogenous pyrogen, the Fe and Zn depressing mediator shows less species specificity than does the endogenous Fe and Zn depressing mediator in serum; this fact may be one of the earliest detectable responses of the host to invasion by infecting micro-organisms.

Further, comparative diurnal changes in Cu, Zn, cholesterol and carotenoids were observed in Leghorn male chicks. NDV involvement in the chicks produced significant alterations in the metabolism of these serum components.

Progress, Part III:

In order to study the effects of infection on host metabolism in a nonhuman primate, we investigated several monkey models in which a gram positive (D. pneumoniae) and gram negative organism (Salmonella typhimurium) host inter-relationship could be examined. The monkeys, restrained in chairs, each had an indwelling catheter in the right atrium of the heart and a thermocouple inserted into back muscles which were connected to a multipoint recorder. In some experiments a plastic window was inserted in the abdomen by the technique of McConnell, et al.[5] By day 3 postsurgery the animals were ready for experimentation.

When a chaired monkey was given an intravenous injection of 10^7 to 10^8 D. pneumoniae (type 1-A, strain 5) organisms, it developed transient rise in body temperature in 1 hr and a 4-5 degree rise in 24 hr which persisted for the next 72-96 hr. The monkeys had normal circadian periodicity in body temperature, high during the day, low at night, which was still present during the febrile phase of illness. During the first 3-8 hr the animals developed leukopenia followed by a marked increase in neutrophiles; the lymphocytes tended to remain low. By 8 hr the monkeys were anorexic, lethargic and might vomit. The clinical illness persisted for 4-5 days after which the body temperature returned to normal and food intake increased.

The addition of a plastic window did not affect the normal development of the infection. Liver samples were obtained from monkeys 24 hr preinfection and 3, 34, 48 and 72 hr postinfection. A 1-2 gm sample of liver was removed at each time period, the window was removed after the last sampling and the monkey was allowed to recover. Histological and biochemical measurements did not reveal any effects due to presence of the plastic window.

Daily blood samples were obtained at 5 different time periods during the pre-exposure period in order to determine baseline concentrations of serum Zn, Fe, and Cu as well as any diurnal variability. A diurnal periodicity was apparent for Fe and Zn, but was not clear-cut for Cu.

Notable changes in the serum concentrations of all 3 metals were observed in both monkeys following exposure to D. pneumoniae. Serum Zn concentrations decreased rapidly, with values well below baseline occurring within 3 hr. Decreases in serum Fe concentrations occurred but maximal depressions were not as rapid. During the first 24 hr, serum Cu values were actually shown to be

depressed below baseline and did not start to increase until the 2nd postexposure day. However, the sampling regime during the first 24 hr included numerous bleedings. This excessive amount of blood sampling probably accounted for the initial decrease in serum Cu.

When the chaired rhesus monkey was given an intravenous (IV) injection of 10^{10} S. typhimurium organisms (either strain ATCC No. 1311 or a MIT strain), it developed a febrile response within 3 hr which persisted in undulating fashion for the next 22 days. The body temperature reached highs of 105-106 F during the day and was normal at night. Over the 22-day period the monkey challenged with ATCC No. 1311 strain had cumulative fever hours (time x degrees > 100 F) of 300 while those infected with the MIT strain had approximately 900 fever hours for the same time period.

With the 1311 strain the monkey had bacteremia that ranged from 10^4-10^7/ml, which persisted for the 22-day period of the experiment. In contrast the 2 monkeys infected with MIT strain had variable bacteremia which rarely reached 10^4/ml and was not present on every day of the infection. Thus, the higher fever index on the MIT strain was not correlated with the level of bacteremia.

The neutrophils were markedly depressed during the early stages of infection and returned to normal concentration by day 10. In contrast the lymphocytes were elevated throughout the course of the infection. The response in white blood cells was similar for both strains tested. Monkeys infected with either strain had a gradual decrease in hematocrit, anorexia, and occasional episodes of vomiting.

Agglutinating antibody titers were determined to the S. typhimurium O antigen in monkeys infected with the MIT strain, both monkeys displayed no agglutinating antibody titers before exposure. By day 8 titers of 1:80 and 1:160 were observed in monkeys J-2-16 and I-7-49 respectively. Monkey J-2-16 maintained a 1:80 titer through day 17; by day 21 the titer had dropped to 1:40. Monkey I-7-49 reached a maximum titer of 1:640 by day 11, and remained at that level until termination of the experiment on day +21.

In response to the IV challenge with S. typhimurium, ATCC 1311, early marked decreases from predetermined baseline values were demonstrated for serum Zn and Fe in both test monkeys. Concentrations were maximally depressed by day 1. Depressions in serum Zn and Fe concentrations appeared to be more severe. In Monkey J-7-89, had the more marked response, with the exception of 2 days the levels remained below baseline throughout the postexposure period. In monkey J-2-44 serum Zn and Fe concentrations returned to baseline by day 7, followed by a progressive increase, which went above baseline values at the termination of the study. This late increase in serum Fe and Zn may have resulted from release of storage Zn and Fe due to tissue necrosis.

Both monkeys displayed early marked increases in serum Cu concentrations with 2-3-fold increases being observed between days 7 and 15. Significant increases in serum Cu were observed as early as day 2 and remained well above baseline throughout the postexposure period.

In response to infection with S. typhimurium, MIT strain, rapid decreases in both serum Zn and Fe were demonstrated in both test animals. Both test animals displayed maximal depressions in both serum Zn and Fe within 8 hr postinfection. During the second week postinfection serum Zn values rose above baseline values in both animals. A corresponding rise, although never going above baseline, in serum Fe values also was observed. Due to the severity of the illness throughout the 21 day postexposure period, these rises in serum Zn and Fe suggest the release of storage Zn and Fe due to tissue necrosis. During the third week of illness, both serum Zn and Fe concentration began to decline steadily, suggesting bodily depletion of these metals.

By contrast, serum Cu concentrations began to increase by day 1; values rose steadily reaching a 3-4-fold increase. A terminal decline from this elevated plateau occurred in 1 monkey.

In monkeys infected with either strain of S. typhimurium, the organisms were first isolated from the stool on days 3-7 and remained positive throughout the study. Upon termination of the experiment, S. typhimurium could be isolated from the liver and spleen and cerebrospinal fluid of both animals and from the lung of one of the monkeys.

Microscopic diagnosis of the tissue from monkeys infected with either strain revealed a resolving bacterial pneumonitis, focal necrotizing hepatitis in two animals, hemopoiesis in the liver of another and meningitis in one of the monkeys infected with the MIT strain. Most of the pathology reflected a long and exhaustive defense effort by the host.

Future studies on host defense mechanism are planned with these monkey models.

Summary, Part III:

Monkey models have been developed to study host interrelationships in gram positive (D. pneumoniae) and gram negative (S. typhimurium) organisms. The former is a mild infection of 4-5 day duration in the monkey, while the latter organism is a more severe infection that persists for > 21 days. Both infections caused similar changes in serum trace metals.

Presentations:

1. Pekarek, R. S. Characterization of the infection-induced endogenous mediator of serum zinc depression. Presented at Federation of American Societies for Experimental Biology, Atlantic City, N. J., 12-17 April 1970.

2. Pekarek, R. S. Endogenous mediator of serum iron depression during infection and endotoxemia. Presented at American Society for Microbiology, Boston, Massachusetts, 26 April - 1 May 1970.

3. Squibb, R. L. Effect of Newcastle disease virus (NSV) on serum diurnal patterns of copper, zinc, cholesterol, and carotenoids in chicks. Presented at the Federation of American Societies for Experimental Biology, Atlantic City, N. J., 12-17 April 1970.

4. Beisel, W. R., R. W. Wannemacher, Jr., R. S. Pekarek, and Peter J. Bartelloni. Presented at American Society for Clinical Nutrition, Atlantic City, N. J., 2 May 1970.

Publications:

1. Pekarek, R. S., K. A. Bostian, P. J. Bartelloni, F. M. Calia, and W. R. Beisel. 1969. The effects of Francisella tularensis infection on iron metabolism in man. Amer. J. Med. Sci. 258:14-25.

2. Pekarek, R. S., and W. R. Beisel. 1969. Effect of endotoxin on serum zinc concentrations in the rat. Appl. Microbiol. 18:482-484.

3. Pekarek, R. S., and W. R. Beisel. 1970. Characterization of the infection-induced endogenous mediator of serum zinc depression. Fed. Proc. 29:297 (abstract).

4. Squibb, R. C., W. R. Beisel, and K. A. Bostian. 1970. Effect of Newcastle disease virus (NDV) on serum diurnal patterns of copper, zinc, cholesterol, and carotenoids in chicks. Fed. Proc. 29:695 (abstract).

5. Pekarek, R. S., and W. R. Beisel. 1970. Endogenous mediator of serum iron depression during infection and endotoxemia. Bact. Proc. p. 81.

6. Beisel, W. R., R. W. Wannemacher, Jr., R. S. Pekarek, and Peter J. Bartelloni. 1970. Early changes in individual serum amino acids and trace metals during a benign viral illness of man. Amer. J. Clin. Nutr. 23:660, (abstract).

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 402:	Development of Methods for the Detection and Assay of Interferon
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Bruno J. Luscri, Ph.D. George W. Jordan, Major, MC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 0L0855	2. DATE OF SUMMARY* 70 07 01		REPORT CONTROL SYMBOL DD-R&E (AR) 636
3. DATE PREV SUM'RY 69 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISS'N INSTR'N DE	8b. SPECIFIC DATA - CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	402
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*
(U) Development of methods for detection and assay of interferon

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	2	30
FISCAL YEAR CURRENT 71	2	30

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Interferon; (U) Viral interference; (U) Sindbis virus; (U) Semliki Forest virus; (U) Chikungunya virus

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Develop tests for use in rapid diagnosis of viral diseases.

24 (U) An interferon of tissue culture origin will be produced and characterized. Standard methods for bioassay will be applied. Interferon is to be produced in quantity and partially purified. Antisera to partially purified interferon are to be tested for possible use in an immunological assay for interferon.

25 (U) 69 07 - 70 06 - Recent advances in interferon research and previous work performed by this unit have been reviewed. The production and assay of chick interferon will be initiated in addition to the continuing work with mouse (L-cell) interferon.

Temporary laboratory space has been obtained for the preparation and titration of the various necessary virus stocks. Semliki Forest and Sindbis viruses have been produced in tissue culture and aliquoted in small amounts for use as challenge agents. Preliminary attempts to induce interferon in chick cells are in progress using Chikungunya virus as the inducing agent.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 402: Development of Methods for the Detection and Assay of Interferon

Description:

Develop methods for the detection and bioassay of interferon.

Progress and Summary:

Recent advances in interferon research and previous work performed under this work unit have been reviewed. The production and assay of chick interferon will be initiated in addition to the continuing work with mouse (L-cell) interferon.

Temporary laboratory space has been obtained for the preparation and titration of the various necessary virus stocks. Semliki Forest and Sindbis viruses have been produced in tissue culture and aliquoted in small amounts for use as challenge agents. Preliminary attempts to induce interferon in chick cells are in progress using Chikungunya virus as the inducing agent.

Publications:

None.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 403:	Separation, Purification and Concentration of Arbovirus Agents and Antigen-Antibody Complexes
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Division:	Virology
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Author:	Neil H. Levitt, Captain, MSC
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OL0856	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISB'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	403
b. [illegible]				
c. [illegible]	CDOG 1212b(9)			

11. TITLE (Precede with Security Classification Code)*

(U) Separation, purification and concentration of arbovirus agents and antigen-antibody complexes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
61 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: NA d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 70	1	5
	CURRENT 71	1	5

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Arboviruses; (U) Immunology; (U) Antigens; (U) Antigen-antibody reactions; (U) Serology; (U) Tissue culture (U) Tobacco mosaic virus; (U) Equine encephalitis (VEE)

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Study arbovirus agents in purified preparations, separate serological or immunological antigens from infectious virus particles, and separate group and specific antigens in order to investigate antigen-antibody complexes in concentrated and/or purified forms.

24 (U) Selected group A arboviruses will be compared as to infective and/or antigenic components. Concentration and purification of viruses will be accomplished by ultrafiltration, column chromatography and density gradient centrifugation. Procedures will be developed to detect specific antibodies to arboviruses using cellulose polyacetate electrophoresis.

25 (U) 69 07 - 70 06 - Studies have been initiated to develop methods for the rapid detection of specific antibodies to arboviruses in human sera. An in vitro procedure employing the cellulose polyacetate electrophoresis technique is presently being developed for visual detection of antigen-antibody reactions. Concentrated preparations of tobacco mosaic virus (TMV) and antiserum to this virus were tested in a model system. Preincubated mixtures of TMV and serum were electrophoresedat 300 V for 60 minutes in veronal buffer, pH 8.6. The migration patterns along the strips were detected by staining with Ponceau red stain. Migration of TMV was inhibited by the presence of immune serum (1:256 or less dilution) as indicated by both a deeply stained precipitate at the point of application (origin) and the absence of a band 3.9 cm from the origin towards the anode, the pattern seen with free TMV. Work is in progress to determine the effectiveness of this procedure using inactivated arboviruses as test antigens.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 403: Separation, Purification and Concentration of Arbovirus Agents and Antigen-Antibody Complexes

Description:

Study arboviruses in purified preparations, separate serological or immunological antigens from infectious virus particles, and separate group and specific antigens in order to investigate antigen-antibody complexes in concentrated and/or purified forms.

Progress:

A study is currently in progress to develop an in vitro procedure for the rapid detection of specific antibody to group A arboviruses in human sera. Due to its rapid and simple operation, the cellulose polyacetate electrophoresis technique was selected for visual detection of specific antigen-antibody reactions [1,2]. Mixtures of concentrated virus (0.025 ml) and 2-fold dilutions of serum (0.025 ml) are incubated in Microtiter plates at 37 C for 60 min. Approximately 4 µl of each reaction mixture is applied to a separate cellulose acetate strip and electrophoresed at 300 V for 60 min; subsequently the strips are fixed and stained with Ponceau red stain followed by destaining with acetic acid.

As a model antigen-antibody system, highly concentrated tobacco mosaic virus (TMV) and specific rabbit antiserum were tested to determine the reliability and sensitivity of this immunoelectrophoretic procedure. Aliquots of TMV and 2-fold dilutions of antiserum were reacted as described previously and electrophoresed. Similar aliquots of TMV were mixed with dilutions of normal rabbit serum and tested concurrently as controls.

Results of this preliminary experiment demonstrated that after electrophoresis, visual differences existed between strips containing reaction mixtures of TMV with immune and normal sera. A deeply stained precipitate was seen at the point of application (origin) for mixtures containing dilutions of immune serum at $\leq$ 1:256. No inhibition of migration was observed for reaction mixtures of TMV and normal rabbit serum at any serum dilution tested.

It has not yet been possible to test this procedure for the detection of specific antibodies to arboviruses due to the unavailability of a highly concentrated killed virus preparation. However, large quantities of

Venezuelan equine encephalitis (VEE) virus are now being concentrated and purified for use as a test antigen in this electrophoretic procedure. This technique, if successful for VEE virus may be useful in the detection of specific antibodies to various groups of animal viruses.

It was anticipated that this cellulose polyacetate electrophoresis procedure described could be modified for the rapid detection of specific viral particles in clinical specimens. By reacting radio-labeled immune Y globulin with an aliquot of VEE virus suspension (similar to the immunoassay for certain hormones [3,4]) attempts were made to detect the presence of virus by evaluating the migration pattern of the labeled globulin. In preliminary experiments ^{125}I-labeled Y globulin was reacted for 1 hr at 37 C with varying dilutions of ultraviolet-light inactivated VEE virus. Mixtures of labeled Y globulin and saline served as controls. Samples were applied to polyacetate strips and electrophoresed for one hour. After electrophoresis, detection of radioactivity along the strip was accomplished by the autoradiography technique.

Differences in migration patterns of experimental and control samples were observed. Further studies are required to evaluate the significance and reproducibility of these results.

Summary:

Studies on the development of an *in vitro* procedure to detect specific antibodies to certain arboviruses in human sera are in progress. Preincubated mixtures of virus and serum have been electrophoresed on cellulose polyacetate strips and reacted with a protein stain. Specific antigen-antibody complexes formed precipitates at the origin and failed to migrate along the strip. This procedure has been used successfully for the detection of antibody to tobacco mosaic virus and awaits further evaluation with arboviruses.

A study employing the cellulose polyacetate electrophoresis procedure and ^{125}I-labeled immune Y globulin is presently in progress to develop methodology for detecting arboviruses in clinical specimens. Migration differences have been observed between free Y globulin and that previously reacted with dilutions of VEE virus. These results are preliminary however, and critical evaluation of this procedure awaits the availability of more data.

Publications:

None.

LITERATURE CITED

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2. Grunbaum, B. W., P. L. Kirk, and W. A. Atchley. 1960. Micro-electrophoresis on cellulose acetate membranes. Anal. Chem. 32:1361-1362.

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ANNUAL PROGRESS REPORT

Project No. 1B662706A096:	Medical Defense Aspects of Biological Agents (U)
Task No. 1B662706A096 03:	Laboratory Identification of Biological Agents
Work Unit No. 096 03 800:	Immunological Studies with Microbial Toxins
Reporting Installation:	U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Maryland
Divisions:	Bacteriology and Animal Assessment
Period Covered by Report:	1 July 1969 to 30 June 1970
Professional Authors:	Virginia G. McGann, Ph.D. (I, II, III) Richard O. Spertzel, Major, VC (II, III) Joseph C. Denniston, Captain, VC (III) Brent J. Rollins, Captain, VC (II) Donald E. Kahn, Captain, VC (III) Elizabeth O. Roberts, Ph.D. (I, II, III)
Reports Control Symbol:	RCS-MEDDH-288(R1)
Security Classification:	UNCLASSIFIED

JOD,DPG

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OL0876	70 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
69 07 01	D. CHANGE	U	U	NA	DE	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62706A	1B662706A096	03	800
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 1212b(9); 1412a(2)			

11. TITLE (Precede with Security Classification Code)

(U) Immunological studies with microbial toxins

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 004900 Defense; 003200 BW, CW, RW

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: NA
d. AMOUNT:
e. KIND OF AWARD:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 70	3	50
FISCAL YEAR CURRENT 71	3	50

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Roberts, E. O. DA

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Enterotoxin, staphylococcal; (U) Immunology; (U) Hemolysins; (U) Antibodies; (U) Toxoid

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Investigate immunologic responses of a susceptible host after exposure to microbial toxin and after immunization with toxoid.

24 (U) Evaluate in vitro methods to determine protective status of antibodies in a susceptible host and to assay for immunogenicity of toxoid preparations. Investigate suitable techniques to compare antigenic composition of a variety of preparations of enterotoxins.

25 (U) 69 07 - 70 06 - Studies were undertaken to potentiate the activity of toxoid prepared from staphylococcal enterotoxin B (SEB) by altering dosage and immunization schedule and by addition of adjuvant. Significant differences were observed in SEB-precipitin response to primary immunization. Fluid toxoid elicited an earlier response than corresponding amounts of alum-precipitated toxoid and at doses of 80 μg N was more effective than at higher or lower doses. Six weeks after immunization with a total dosage of 100 μg N, given in 1, 2 or 3 injections, no differences were observed in antibody response or protection. Although enterotoxins and toxoids prepared from purified material can be assayed readily in vitro, there is no satisfactory method for estimating toxoid content of formalinized culture filtrates. Hemolysins in SEB preparations were highly antigenic but did not contribute to toxicity in monkeys. Investigations are in progress on serological aspects of the response of monkeys to reexposure with SEB, and studies were initiated on type A staphylococcal enterotoxin.

Purified and crude SEB preparations can be neutralized by equine antitoxin.

Reexposure of monkeys to SEB often resulted in an anaphylactic-like syndrome.

*Available to contractors upon originator's approval.

DD FORM 1498-1 (FOR ARMY USE)

BODY OF REPORT

Project No. 1B662706A096: Medical Defense Aspects of Biological Agents (U)

Task No. 1B662706A096 03: Laboratory Identification of Biological Agents

Work Unit No. 096 03 800: Immunological Studies with Microbial Toxins

Description:

Investigate immunologic response of a susceptible host after exposure to microbial toxin and after immunization with toxoid.

Progress, Part I:

Evaluation of *in vitro* methods for estimating resistance to intoxication with staphylococcal enterotoxin B (SEB) continued and studies were initiated with enterotoxin A (SEA). Investigations are in progress on techniques for measuring antigenic components in a variety of preparations of enterotoxins and their toxoids, for detecting SEB in biologic fluids and for predicting response to repeated exposures with enterotoxin.

Formalin-treated toxoids. Antigenic and protective properties of a production lot of formalin-treated SEB toxoid, Lot 87285, received from Chas. Pfizer & Co., Inc. (Contract No. DADA 17-68-C-8079) were summarized in a previous report.[1/] Further studies were concerned with determining conditions for increasing efficacy of toxoid.

The effect of injection schedule and dosage on antibody response and protection was investigated. Animals received a total dose of 100-150 µg toxoid N given in 1-3 subcutaneous (SC) injections; selection of the total dose was regulated by earlier studies on safety and efficacy. A detailed description of experimental procedures is reported under Work Unit No. 096 02 007. Early antibody response to immunization, particularly toxin-precipitin response, was markedly affected by dosage (Table I). Essentially no SEB-precipitating antibodies appeared within 6 weeks of a single injection of 50 µg N. After the 80 µg N dose, 10 of 12 monkeys developed SEB-precipitins: 1 in 2 weeks, an additional 6 by 4 weeks, and 3 at 6 weeks; 2 monkeys that were positive at 4 weeks had no detectable precipitins at 6 weeks. The antibody response to a single injection of 100 µg N developed slowly but by 6 weeks both incidence of response and titers compared favorably with the post-booster response of other groups. No advantage was gained in protection or antibody response when the same amount of antigen was divided among 3 injections.

TABLE I. TOXIN-PRECIPITIN RESPONSE TO IMMUNIZATION WITH SEB TOXOID, LOT 87285

TOXOID			NUMBER ANTIBODY-POSITIVE/NUMBER IMMUNIZED				
Primary Dose	Booster Dose		After Initial Injection (Weeks)				After Booster
µg	µg N	Time	0	2	4	6	6 Week
100			0/6	0/6	1/6	5/6	
80	20	4 weeks	0/6	1/6	5/6		5/6
		6 weeks	0/6	0/6	2/6	3/6	3/6
50	50	4 weeks	0/18	0/18	0/18		14/18
		6 weeks	0/6	0/6	0/6	1/6	5/6

The presence of SEB-precipitating antibody correlated well with protection against the lethal effect of challenge with 300 µg SEB/kg. Animals that succumbed to challenge lacked SEB-precipitins whereas 28 of 31 survivors had precipitating antibody. Protection against the emetic effect of a 10 µg/kg challenge with 30% pure SEB, Lot 52-68, did not correlate so well with antibody; illness occurred in each of 2 monkeys that lacked SEB-precipitins as well as in 2 of 4 with precipitins.

Protection induced by immunization with alum-precipitated toxoid (Lot 87285) was the same as that with fluid toxoid (see Work Unit No. 096 02 007). SEB-precipitins, however, appeared more rapidly after immunization with the fluid product; within 2 weeks after injection 7 of 24 monkeys responded to fluid toxoid in contrast to 2 of 24 to alum-precipitated toxoid (Table II). There was some indication that immunity might persist longer after immunization with alum-precipitated material. At 10 weeks, incidence of SEB-precipitating antibody was increasing in the alum-precipitate group and was decreasing in the fluid toxoid group. All monkeys without antibody and 1 of 44 with antibody responded to challenge.

TABLE II. SEB-PRECIPITIN RESPONSE TO IMMUNIZATION WITH FLUID OR ALUM-PRECIPITATED SEB TOXOID, LOT 87285

TOXOID	CHALLENGE GROUP (μg/kg)	NO. ANTIBODY POSITIVE/NO. IMMUNIZED By Week After First Injection					CHALLENGE RESPONSE
		0	2	4	7	10	R/T[a/]
Fluid	10	0/12	4/12	4/12	12/12	11/12	1/12
	300	0/12	3/12	3/12	12/12	11/12	1/12
Alum-precipitated	10	0/12	1/12	1/12	11/12	11/12	2/12
	300	0/12	1/12	1/12	8/11	11/11	0/11

a/ R/T = At 10 μg/kg = $\frac{\text{Number ill}}{\text{Number tested}}$; at 300 μg/kg = $\frac{\text{Number dead}}{\text{Number tested}}$

Other indices of antibody response, hemagglutinins (HA) and toxoid precipitins, showed poor correlation with protection. Essentially all monkeys had toxoid precipitins within 2 weeks after a single injection of toxoid. Only 60% of the monkeys had HA titers at 2 weeks and although incidence of response increased to 90% by 4 weeks, the median titer was only 1:40. High titers (>1:80) appeared only after booster injection. Approximately 11% of the monkeys with early toxoid-precipitins never developed SEB-precipitins. Persistence of SEB-precipitins was not related to that of other antibodies. Consequently, after toxoid immunization no other antibody test could be used to predict SEB-precipitin response.

Characterization of toxin and toxoid preparations. The use of 30% pure SEB, Lot 52-68, for challenge and the proposal to use detoxified whole culture filtrates for immunization (see Work Unit No. 096 02 800) produced a requirement for detection and quantitation of the biologically-active extracellular products of Staphylococcus. Initial studies were restricted to preparations of SEB, SEA and hemolysins because highly purified reference material was available for the enterotoxins and α-, β- and δ-lysins were detected readily by hemolysis.

Reactions of equine antitoxin produced with SEB, Lot 14-31, indicated that even highly purified SEB contained traces of non-specific antigens. At least 5 lines of antigen-antibody reaction were demonstrated with culture filtrates of SEB, as well as low-titering reactions with purified SEA. Antibodies for these antigens appeared only after repeated injection with large amounts of purified SEB (1-3 gm). Purified SEA had at least 2 antigenic components that reacted with the equine anti-SEB serum and with anti-SEA sera.

Direct titration for hemolysins indicated that Lot 14-30 SEB contained 4 units of α-lysin and 2 units of β-lysin/mg SEB. Lot 52-68 contained 200 units of α-lysin/mg SEB and little or no β-lysin. No lysins were detected in purified SEA at concentrations of 100 µg SEA/ml. Identification of α-hemolysin by precipitin reactions is not yet possible because of lack of purified reference material. Twenty-four monkeys were surveyed for the presence of naturally-acquired antibody for α-hemolysin. Sera of all animals could neutralize 2 units of lysin/ml serum; 30% could neutralize 10 units/ml and 50%, at least 25 units/ml. No increase in antilysin occurred after immunization with SEB-toxoid, and protection against challenge with Lot 52-68 SEB was unrelated to antilysin activity. Survivors of challenge, however, showed a rapid 10-fold increase in antilysin, often without showing any increase in anti-SEB.

Radial immunodiffusion techniques have been developed to quantitate purified enterotoxins, toxoids prepared from purified enterotoxin and known toxic components in crude culture filtrates. No method has yet been satisfactory for identifying and quantitating enterotoxoid in formalin-treated filtrates. Detoxified filtrates gave weak reactions with anti-enterotoxoids and the nature of the reaction suggested that a component other than enterotoxoid, possibly detoxified α-hemolysin, was involved. On the basis of serology, formalin treatment of filtrates appeared to destroy over 90% of the antigenic activity. Efficacy tests with detoxified filtrates are now in progress and should provide a better basis for proceeding with serologic studies.

Naturally-acquired antibody. The efficacy of naturally-acquired antibody in protection against SEB was reported elsewhere.[2/] Studies were initiated to determine the incidence of naturally-acquired SEA hemagglutinins in man and monkeys. In a group of 50 people, 40% had SEA titers of 1:10-1:40 and 55%, SEB titers of 1:20-1:5120; 25% had antibody for both SEA and SEB and 30% had antibodies to neither enterotoxin. Low-titering SEA antibody, 1:10-1:20, was present in sera from 7 of 195 monkeys. After IV challenge with $\leq$0.5 µg SEA/kg, 1 of 7 antibody-positive and 6 of 153 antibody-negative monkeys died. Antibody-positive survivors showed no booster reaction but 7 of the antibody-negative survivors developed titers of 1:10-1:640.

Very few sera have been tested for SEA-precipitins. Serial bleedings were available from 27 individuals involved in an outbreak of food poisoning; 4 of these individuals had strong SEA-precipitating antibody. Simultaneous titration for HA revealed that some strong precipitating sera had no HA-antibody and HA titers, when present, never exceeded 1:80, suggesting that optimum conditions for hemagglutination have not been developed.

Reexposure studies. A cooperative study with personnel at Fort Detrick on response of monkeys to reexposure with SEB has been reported.[2/] The results indicated that a number of animals with high levels of prechallenge antibody had severe reactions to respiratory challenge; the symptoms resembled those usually associated with hypersensitivity. Investigations have continued

on various parameters of response of SEB-experienced monkeys after reexposure by the IV route.

Preexposure sera and sera from serial bleedings obtained 15 min - 48 hr after challenge were examined for antibody content and circulating SEB. HA and precipitin titers were unsatisfactory indices of potential for inactivating the challenge dose, but by adding a known quantity of SEB to serum it was possible to determine the SEB-binding capacity. The relationship of bound SEB/ml to the theoretical value for challenge SEB/ml was used to indicate antibody competence.

The initial rate of clearance and persistence of antibody-precipitable SEB in the circulation after challenge with 500 μg/kg was related to antibody competence (Table III). In nonexperienced, antibody-negative monkeys, 60% of the challenge dose was cleared within 10 min and 80% within 20 min, but occasionally 10% - 20% remained in the circulation for 1½ hr. In sera from 5 of 7 experienced monkeys capable of inactivating the challenge dose (100% competence), no SEB was detected after challenge; the other 2 monkeys behaved like nonexperienced animals. At decreasing levels of competence, the initial rate of clearance was comparable to that of nonexperienced monkeys but SEB persisted in the circulation for a significantly longer time. In 50% of these animals, SEB remained in the serum for at least 4 hr.

TABLE III. ANTIBODY COMPETENCE[a] AND RESPONSE TO IV CHALLENGE OF SEB-EXPERIENCED MONKEYS

GROUP	ANTIBODY COMPETENCE %	NO.	MEAN DURATION OF CIRCULATING SEB (HR)	NO. WITH OVERT RESPONSE	
				Immediate Symptoms[b]	Death[c]
No Experience:	0	5	3/4	0	5
Antibody-Negative					
Experienced:	100	7	<1/2	5	1
Antibody-Positive	25-90	11	3-3/4	9	4
	<25	1	4	1	0

a. Ability of prechallenge serum to inactivate the challenge dose; expressed as percent of challenge dose inactivated.

b. Facial flush, vomition or respiratory distress ≤5 min after challenge.

c. Deaths of nonexperienced monkeys occurred after 36 hr; deaths of experienced monkeys occurred at 3/4 - 12 hr.

It is doubtful that circulating-SEB was combined with antibody because precipitation with anti-monkey serum did not remove SEB activity. Many sera containing circulating SEB were able to inactivate SEB added in vitro, suggesting the simultaneous presence of antigen and antibody and the possibility that in vivo alteration of SEB may block combination with some antibodies.

Antibody activity of all animals decreased precipitously $\leq$ 15 min after challenge. Approximately 80% of the original activity was restored within 3-6 hr and 100%, within 24-48 hr. At time of death, sera obtained from experienced monkeys were capable of inactivating added SEB at levels equivalent to 25-50% competence, indicating that significant amounts of antibody were present.

Estimates of antibody competence probably were better than precipitin or HA reactions for reflecting total antibody content but they could not be used to predict the immediate response to challenge. If hypersensitivity is involved, IgE antibody determinations may be required to indicate the sensitized state.

Summary, Part I:

The state and dosage of SEB toxoid markedly influence the SEB-precipitin response to primary immunization but have significantly less effect on response to subsequent immunization. Fluid toxoid elicited earlier antibody production than corresponding amounts of alum-precipitated toxoid, and a single dose of fluid toxoid containing 80 μg N was more effective than higher or lower doses. The SEB-precipitin response to a single dose of 100 μg N developed more slowly, but within 6 weeks, was equivalent to the response produced by the same amount of toxoid divided among 2 or 3 injections. Toxin precipitation continues to give the best index of protection; other serological reactions are not effective substitutes.

Studies were initiated to detect biologically-active extracellular products of staphylococcal culture filtrates. Purified and crude enterotoxins and enterotoxoids prepared from purified material can be measured with radial immunodiffusion techniques, but no satisfactory method is yet available for detecting toxoid in formalinized filtrates. Although most enterotoxin preparations and filtrates contain hemolysins, their anti-hemolysins to not contribute to protection against crude SEB. Preliminary results suggest that modification of HA procedures will be required to detect antibodies for SEA.

Antibody-positive, experienced monkeys often respond to SEB exposure with symptoms that resemble hypersensitivity. Techniques for predicting severe overt response are under investigation. Many monkeys that appear to have sufficient antibody to neutralize the challenge dose demonstrate immediate physiologic response to SEB, but the most severe reactions occur in animals with partial neutralizing activity. Serum antibody always decreases precipitously immediately after challenge but soon returns to original levels. Animals that can partially neutralize the challenge dose may have SEB in

their circulation for as long as 6 hr; circulating SEB and antibody activity are often found in the same serum sample. Sera from all experienced animals that succumbed to challenge demonstrated antibody activity at time of death.

Progress, Part II:

The protective capacity of antitoxin prepared against highly purified SEB (Lot 14-30) was evaluated for 30% pure SEB (Lot 52-68). Mixtures of enterotoxin and antitoxin at equivalence and at 1.5 times equivalence were tested for toxicity in monkeys. Solutions containing 1,000 µg SEB/ml were mixed with the appropriate amount of equine antitoxin, held at 4 C for 18 hr with occasional shaking, and then centrifuged to remove the precipitate. The injection dose was that amount of supernatant that would have contained 1,000 µg/kg of toxin if no neutralization had occurred. Antitoxin controls were injected IV with the appropriate amount of equine antitoxin; toxin controls received 1,000 µg/kg of either Lot 14-30 or 52-68. One of 3 control monkeys became ill immediately after injection of equine antitoxin. Consequently, all experimental challenges were by the subcutaneous (SC) route.

All toxin control animals died; all monkeys receiving toxin-antitoxin mixtures survived (Table IV). No overt symptoms appeared except in the group that received Lot 14-30 and antitoxin at 1.5 equivalence. The time required for symptoms to appear, 34 min and 8 hr, respectively, would suggest that reaction to serum protein was not implicated in the response of these animals.

TABLE IV. IN-VITRO SEB TOXIN-ANTITOXIN NEUTRALIZATION IN MONKEYS (3/GROUP) SC INOCULATION

INJECTION MIXTURE	NO. SICK	NO. DEAD
Equine antitoxin, IV	1	0
Lot 14-30 + antitoxin, equivalence	0	0
Lot 14-30 + antitoxin, 1.5 equivalence	2	0
Lot 14-30 + physiologic NaCl	3	3
Lot 52-68 + antitoxin, equivalence	0	0
Lot 52-68 + antitoxin, 1.5 equivalence	0	0
Lot 52-68 + physiologic NaCl	3	3

a. Immediate response suggesting reaction to foreign protein.

Summary, Part II:

Both purified and crude preparations of SEB can be neutralized by equine antitoxin at equivalence.

Progress, Part III:

On occasion, early deaths occurred in SEB-experienced primates on re-exposure to high levels of SEB (500 µg/kg). Studies were initiated to investigate the possibility that immediate hypersensitivity reactions or the activity of antigen-antibody aggregates were involved in the early-death response. In preliminary studies, 8 guinea pigs received a sensitizing dose of 1,000 µg SEB. Eight weeks later these animals received a shocking dose of 100 µg SEB. Seven animals developed classical signs of immediate type anaphylactic reactions. This preliminary data, coupled with current studies on SEB skin hypersensitivity in the primate, reemphasized the sensitizing potential of SEB.

Monkeys selected for investigation of the pathophysiology of primate response had survived IV exposure to SEB, either with or without prior immunization with toxoid, or had survived both IV and respiratory exposure. Their HA titers ranged from 1:640 - 1:320,000 and their precipitin titers from 0 - 1:32. Table V summarizes clinical data from this study.

TABLE V. CLINICAL SIGNS OF SEB-EXPERIENCED M. MULATTA: CHALLENGE DOSE 500 µg/kg IV, SEB 1418-B1

RESPONSE	INCIDENCE OF RESPONSE	% OF RESPONSE
Emesis 1-2 min postchallenge	8/20	40
Emesis/retching 1-5 min postchallenge	14/20	70
Distress 1-10 min postchallenge	19/20	95
Facial flush 1-5 min postchallenge	10/20	50
Mortality	4/20	20
Hypotension 1-10 min postchallenge	6/8	75
Tachycardia 1-10 min postchallenge	6/8	75
Increased respiratory frequency	8/8	100
Leukopenia 1-10 min postchallenge	9/9	100
Absolute neutropenia 1-10 min postchallenge	7/9	78

The early development of emesis, distress, facial flush, hypotension, tachycardia, increased frequency of respiration, leukopenia, and neutropenia are classical signs of anaphylaxis in the rhesus monkey.[3-5] However, thrombocytopenia, a consistent finding in one of these reports,[5] was not observed in this study.

To substantiate the hypothesis that facial flushing was related to vasodilation and vascular leakage, 6 SEB-experienced monkeys were given 50 mg Evans Blue autoconjugated in 5% human serum albumin 15 min prior to challenge. Three animals developed facial flushing 1-5 min postchallenge, facial tissues remained stained for 3-4 days. This is indicative of true increased vascular permeability and vascular leakage often associated with histamine release in immediate hypersensitivity.

Weiszer et al[6] indicated that with Ascaris antigens, there is a relationship between the skin sensitivity of rhesus monkeys and their susceptibility to anaphylactic shock. The existence of such a relationship between cutaneous hypersensitivity and the anaphylactic shock-like syndrome seen in some SEB-experienced monkeys upon systemic challenge with toxin is currently under investigation.

To date, 4 skin-reactive monkeys have been challenged. The results are presented in Table VI. Available data suggest that the cutaneous reactivity of a monkey can be used to predict the susceptibility of the animal to the anaphylactic syndrome. Other animals have been skin-tested for further experimentation.

TABLE VI. SUSCEPTIBILITY OF SEB-SKIN-SENSITIVE RHESUS MONKEYS TO IV CHALLENGE WITH 500 µg/kg SEB AT 3 MON.

MONKEY NO.	PRECIPITIN TITER DAY 0	SKIN REACTIVITY TO SEB DAY 0	ANAPHYLACTIC-LIKE RESPONSE TO CHALLENGE
J4-3	1:1	+	+ and death
J4-83	1:1	+	+
J4-25	>1:32	+	+
J4-30	>1:32	±	Weak +

Summary, Part III:

A study has been initiated to define the pathophysiology of deaths in SEB-immune animals when subsequently challenged with SEB. Data thus far are clearly indicative of an immediate hypersensitivity reaction -- anaphylactic in nature. Additionally, the cutaneous reactivity of a monkey may be useful in predicting the susceptibility of the animal to the anaphylactic syndrome.

Publications:

None

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2. Commission on Epidemiological Survey. December 1969. Annual Report to the Armed Forces Epidemiological Board, FY 1969. p. 21 to 33.

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AUTHOR INDEX

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APPENDIX A

U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES GUEST LECTURE SERIES

DATE	GUEST LECTURER	TITLE OF PRESENTATION
23 Oct 69	Colonel Irvin C. Plough, MC Commanding Officer U. S. Army Medical Research and Development Command Washington, D. C.	Medical Research and the Military Mission.
20 Nov 69	Dr. Malcolm S. Artenstein Chief, Department of Bacteriology Division of Communicable Disease and Immunology Walter Reed Army Institute of Research Washington, D. C.	Development of a Meningococcal Vaccine.
18 Dec 69	Dr. Herbert L. Dupont University of Maryland School of Medicine Baltimore, Maryland	Study of Shigella Vaccines in Man.
22 Jan 70	Dr. E. C. Sharman Staff Assistant Emergency Programs Agricultural Research Service U. S. Department of Agriculture Washington, D. C.	Foot and Mouth Disease Outbreak in England.
5 Mar 70	Dr. Ralph Snyderman, M.D., and Dr. Thomas Robert Tempel, D.D.S. National Institute of Dental Research National Institutes of Health Bethesda, Maryland	Mediators of Neutrophil Chemotaxis.
2 Apr 70	Lt Colonel Daniel G. Sheehan, MC Chief, Department of Experimental Pathology Walter Reed Army Institute of Research Washington, D. C.	Histochemistry of the Small Intestine in SEB Intoxication.
30 Apr 70	Dr. Donald L. Williams Chief Staff Veterinarian Screw Worm Eradication Program Animal Health Division U. S. Department of Agriculture Hyattsville, Maryland	Screw Worm Eradication.

DATE	GUEST LECTURER	TITLE OF PRESENTATION
21 May 70	Dr. Joseph A. Bellanti Associate Professor of Pediatrics and Microbiology Georgetown University Medical Center Washington, D. C.	Phagocytosis, Molecular Aspects.

APPENDIX B

U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES PROFESSIONAL STAFF MEETINGS

DATE	LECTURER	TITLE OF PRESENTATION
26 Sep 69	Dr. Robert S. Pekarek Physical Sciences Division	Trace Metal Metabolism during Infection and Endotoxemia.
	Dr. Anne Buzzell Physical Sciences Division	Metal Chelation and its Application to Biological Membrane Theory.
	Dr. Robert W. Wannemacher, Jr. Physical Sciences Division	Significance of Serum Free Amino Acid Changes during Infection.
	Captain Michael C. Powanda, MSC Physical Sciences Division	Typhoid, Typhimurium, and Tryptophan.
	LT Robert P. Nalewaik, USNR Physical Sciences Division	RNA Metabolism in Mycoplasma.
	Lt Colonel Kenneth A. Woeber, MC Chief, Physical Sciences Division	Alterations in Thyroid Hormone Economy in the Primate during Acute Pneumococcal Infection.
31 Oct 69	Mr. Eugene D. Massey Bacteriology Division	Microtitration of Tularemia Agglutinins.
	Mrs. Mary H. Wilkie Bacteriology Division	Resistance of Protein Antigen in Immune Complexes to Pronase Digestion.
	Dr. Virginia G. McGann Bacteriology Division	Evaluation of Immunity against Staphylococcal Enterotoxin B.
21 Nov 69	Major George W. Jordan, MC Virology Division	Interferon.
	Major David M. Robinson, VC Virology Division	A Review of Q Fever Vaccines.
	Lt Colonel Robert W. McKinney, MSC Chief, Virology Division	Activities Relative to Venezuelan Equine Encephalitis Epizootic in Central America.

DATE	LECTURER	TITLE OF PRESENTATION
30 Jan 70	Lt Colonel Peter J. Bartelloni, MC Chief, Medical Division	Studies on Arbovirus Vaccines.
	Major John L. Winnacker, MC Medical Division	Growth Hormone Response to Pneumococcal Infection in the Monkey.
	Major Theodore S. Herman, MC Medical Division	The Effect of Pneumococcal Infection on Tyrosine Transaminase.
27 Feb 70	Captain J. Brent Rollins, VC Animal Assessment Division	Electrophoretic Patterns in Immune and Nonimmune Beagle Dogs Infected with Either Virulent or Attenuated ICH Virus.
		Review of Staphylococcal Enterotoxin B Toxoid.
	Captain Jerry D. Weil, VC Animal Assessment Division	Natural Occurring Shigellosis in Monkeys.
	Captain Joseph C. Denniston, Jr.,VC Animal Assessment Division	Enterotoxin Challenge of Experienced Monkeys.
27 Mar 70	Colonel Joseph F. Metzger, MC Chief, Pathology Division	Purification of Enterotoxins of Staphylococcus aureus
	Captain Ulysses McElyea, Jr., VC Pathology Division	Quantitative Pharmacology of SEB in the Primate.
	Captain Garrett S. Dill, Jr., VC Pathology Division	Incidental Pathologic Lesions in the Primate Colony.
	Captain James W. Stiles, MSC Pathology Division	Ultrastructural Changes of Monkeys Chronically Infused with SEB.
24 Apr 70	Captain William H. Habig, MSC Microbiology Division	The Cytochromes of Pasteurella pestis.
	Mr. Daniel N. Harrison Microbiology Division	Pseudolysogeny of Pasteurella pestis.
	Dr. James H. Rust, Jr. Department of Bacterial Diseases Div of Comm Disease & Immunology Walter Reed Army Institute of Res	Epidemiological Implications of Cannibalism in the Transmission of Plague.

DATE	LECTURER	TITLE OF PRESENTATION
22 May 70	Dr. William R. Beisel, M.D. Scientific Advisor	Panel presentation: Use of Subhuman Primates in Infectious Disease Research.
	Colonel John D. Marshall, Jr., MSC Chief, Microbiology Division	
	Major Richard O. Spertzel, VC Chief, Animal Assessment Division	
	Major Jerry S. Walker, VC Virology Division	
	Major John L. Winnacker, MC Medical Division	
	Major Gordon L. Bilbrey, MC Medical Division	
	Captain James B. Walford, III, VC Physical Sciences Division	

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APPENDIX C

U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES
FORMAL PRESENTATIONS AND BRIEFINGS

Date and Group or Individual	Individual(s) Participating	Subject
1 Jul 69 Summer Study Meeting in the Microbiological Problems of Manned Space Flight, Woods Hole, Massachusetts	Dr. William R. Beisel, M.D.	Participant in the Study Panel "Early Detection Methods"
6 Jul 69 Research Staff of Plum Island Animal Disease Laboratory U. S. Department of Agriculture Greenport, Long Island, New York	Major Jerry S. Walker, VC	Rift Valley Fever.
8 Jul 69 Global Medicine Course Walter Reed Army Institute of Research	Lt Colonel John D. Marshall, Jr., MSC	Plague.
8 Jul 69 Dr. Herbert Pollock and Dr. Donald Sheldon Institute for Defense Analyses Arlington, Virginia	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D.	Metabolic Mechanisms of Infection.
24 Jul 69 National Communicable Disease Center Atlanta, Georgia	Colonel Dan Crozier, MC	Participant in conference on VEE Epizootic in Central America.

JOD,DPG

Date and Group or Individual	Individual(s) Participating	Subject
5 Aug 69 Colonel William J. Lynch, Colonel Donald L. Howie, Major Curtis G. Unger, and Dr. Francis W. Worthland Army Research Office, Washington, D.C.	Colonel Dan Crozier, MC	Medical Defense Aspects of BW.
13 Aug 69 Dr. William J. Peeples Maryland Commissioner of Health	Dr. William R. Beisel, M.D.	Medical Aspects of BW Defense.
13 Aug 69 Commander James W. Fresh Naval Medical Research Unit #1 Berkeley, California	Dr. William R. Beisel, M.D.	Medical Defense Against BW.
14 Aug 69 National Communicable Disease Center Atlanta, Georgia	Colonel Dan Crozier, MC	Participant in conference on VEE Epizootic in Central America.
14 Aug 69 Lt Colonel John E. Hill, Dr. Chris J. D. Zarafonetis Medical Consultants' Office Office of The Surgeon General, DA	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D. Lt Colonel Peter J. Bartelloni, MC	Medical Defense Aspects of BW and tour of new medical facility.
21 Aug 69 Brigadier General Carl W. Hughes, MC Commanding General Walter Reed General Hospital	Colonel Dan Crozier, MC	Orientation briefing and tour of new medical facility.
29 Aug 69 Third International Biophysics Congress Cambridge, Massachusetts	Dr. Anne Buzzell	Ultracentrifugal Analysis of Metal Chelation by Polyvinyl Toluene Latex (PVL) Spheres.

Date and Group or Individual	Individual(s) Participating	Subject
4 Sep 69 Commission on Epidemiological Survey, Armed Forces Epidemiological Board	Colonel Dan Crozier, MC	Medical Defense Against BW.
	Dr. Francis E. Cole, Jr.	Sequential Immunization - Group A Arboviruses.
	Lt Colonel Peter J. Bartelloni, MC	Clinical Studies - Equine Encephalitis Vaccines.
	Colonel Joseph F. Metzger, MC	Staphylococcal Enterotoxin B (SEB) Characteristics of SEB Preparations.
		Methods for Augmentation of SEB Immunity.
	Dr. Virginia G. McGann	Evaluation of Immunity Against SEB.
	Captain J. Brent Rollins, VC	SEB Toxoid.
	Dr. William R. Beisel, M.D.	Mechanisms of SEB Antibody Protection.
	Lt Colonel John D. Marshall, Jr., MSC	Studies on Plague Vaccines.
	Mrs. Mary H. Wilkie	Resistance of Protein Antigen in Immune Complexes to Pronase Digestion.
	Lt Colonel Robert W. McKinney, MSC	Report on Equine Encephalitis Epizootic in Central America.

Date and Group or Individual	Individual(s) Participating	Subject
5 Sep 69 Commission on Epidemiological Survey, Armed Forces Epidemiological Board	Lt Colonel Peter J. Bartelloni, MC	Clinical Studies of Tularemia Vaccine.
	Major Jerry S. Walker, VC	Other Vaccines and Diagnostic Reagents.
10 Sep 69 Mr. Gerald Astor LOOK Magazine Representative	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D.	Interview arranged through DOD channels on Medical Defense Aspects of BW.
11 Sep 69 Elder Clark Smith, Director National Service Organization of General Conference of Seventh-day Adventists, Washington, D. C. Accompanied by a group of Conference Officials	Colonel Dan Crozier, MC Major Robert A. Massey, MSC	Briefing on Project WHITECOAT.
15-19 Sep 69 Third International Symposium on Aerobiology, Brighton, England	Colonel Joseph F. Metzger, MC	Rapid Diagnosis in Biological Warfare Defense.
17 Sep 69 Group of Chaplains from the National Service Organization of General Conference of Seventh-day Adventists	Colonel Dan Crozier, MC Major Robert A. Massey, MSC	Briefing on Project WHITECOAT
23 Sep 69 Annual Short Course: Pathology of Laboratory Animals Armed Forces Institute of Pathology	Lt Colonel Gilberto S. Trevino, VC	Participant as faculty member in course: Parasites of the Monkey.
	Major Jerry S. Walker, VC	Clinical Appearance of Idiopathic Hemorrhagic Syndrome in Military Dog

Date and Group or Individual	Individual(s) Participating	Subject
25 Sep 69 Agricultural Research Service, Animal Health Division, U. S. Department of Agriculture Hyattsville, Maryland	Colonel Dan Crozier, MC	Recent outbreaks of VEE; Epizootology & Control Methods in Colombia.
	Major Richard O. Spertzel, VC	Recent outbreaks of VEE in Guatemala and El Salvador.
30 Sep 69 Seminar, Department of Biochemistry, State University of New York, Buffalo, New York	Dr. William R. Beisel, M.D.	Tryptophan Metabolism during Infection.
30 Sep 69 Luncheon Presentation to Clinical Laboratory Investigators, Buffalo County Hospital, Buffalo, New York	Dr. William R. Beisel, M.D.	Diagnostic Changes during early Infection.
7 Oct 69 Current Trends in Medical Laboratory Activities Course, Walter Reed Army Institute of Research	Dr. William R. Beisel, M.D.	Early Diagnosis of Infection.
9 Oct 69 Colonel F. S. Leitnaker U.S. Army Materiel Command Surgeon Washington, D. C.	Colonel Dan Crozier, MC	Medical Research Activities of USAMRIID.
14 Oct 69 Mr. Douglas Chevalier Washington Post Photographer	Colonel Dan Crozier, MC	Interview approved through DOD channels on Medical Defense Against BW.

Date and Group or Individual	Individual(s) Participating	Subject
13 Oct 69 20th Annual Session, American Association for Laboratory Animal Science, Dallas, Texas	Captain Frank E. Chapple, III, VC	Surgical Technique for Cross-circulation of Rhesus Monkeys.
	Captain Jerry D. Weil, VC	Incidence of Shigella Species in Clinically Healthy Monkeys.
	Captain J. Brent Rollins, VC	Hematologic Studies of the Rhesus Monkey (Macaca mulatta)
20 Oct 69 Mr. John Silver Aerobiology Research Establishment, Porton Downs, England	Dr. William R. Beisel, M.D.	Physiology of SEB Toxicity.
20 Oct 69 American Association of Poison Control Centers, Chicago, Illinois	Captain Robert H. Fiser, MC	Use of Activated Charcoal in Barbiturate and Glutethimide Poisonings.
21 Oct 69 Major Claudien Richard Jos. Pellerin Canadian Army Ofc of Staff Director, Scientific Technical Intelligence Medical, Canadian Forces, HQ, Ottawa, Canada; and Mr. Harry Lucia, Medical Intelligence Ofc, Office of The Surgeon General.	Colonel Dan Crozier, MC	Medical Intelligence Requirements of BW Defense.
21 Oct 69 Mr. Richard Lebherz Reporter, Frederick News-Post	Colonel Dan Crozier, MC	Interview cleared through channels on medical research activities of USAMRIID.

Date and Group or Individual	Individual(s) Participating	Subject
23 Oct 69 83d Joint Medical Research Conference, Office of Director, Defense Research and Engineering, Pentagon, Washington	Dr. William R. Beisel, M.D.	Metabolic Approaches to the Rapid Diagnosis of Infectious Illness.
24 Oct 69 Armed Forces Epidemiological Board Meeting	Colonel Dan Crozier, MC	Presentation of report of the Commission on Epidemiological Survey to the Board.
24 Oct 69 Dr. Thomas Edward Baldwin Keen Head, Physiology Group, Defence Standards Laboratories, Armadale, Victoria, Australia	Dr. William R. Beisel, M.D.	Medical Research Activities of USAMRIID.
24 Oct 69 Professor L. Joe Berry Chairman, Department of Biology, Bryn Mawr College	Dr. William R. Beisel, M.D.	Role of Infection in Tryptophan Metabolism.
27 Oct 69 Representatives of Public Health Service Emergency Health Services Div, and of the Environmental Control Administration	Colonel Dan Crozier, MC	Medical Aspects of Defense.
30 Oct 69 Commission on Rickettsial Diseases, Armed Forces Epidemiological Board, and Ad Hoc Committee on Q Fever, Walter Reed Army Institute of Research.	Colonel Dan Crozier, MC Dr. Francis E. Cole, Jr. Major David M. Robinson, VC	Participants in meeting of the Commission and as members of the Ad Hoc Committee on Q Fever. M-44 Strain of Q Fever Vaccine.

Date and Group or Individual	Individual(s) Participating	Subject
1 Nov 69 U. S. Army Medical Department Veterinary School, Chicago, Illinois	Major Jerry S. Walker, VC	Tropical Canine Pancytopenia.
4 Nov 69 Lieutenant General Hal B. Jennings, MC The Surgeon General, Dept of the Army; Colonel Irvin C. Plough, Colonel Kenneth R. Dirks, Lt Colonel Howard D. Gutin of U.S. Army Medical R&D Command	Colonel Dan Crozier, MC Lt Colonel Benjamin B. Kirkland, MC Lt Colonel Robert W. McKinney, MSC Major Robert A. Massey, MSC	Visit and tour of USAMRIID facilities.
12 Nov 69 Staff members of Defense Intelligence Agency, Washington, D. C.	Lt Colonel Robert W. McKinney, MSC	VEE in Central America.
14 Nov 69 Annual American Thyroid Association Meeting Chicago, Illinois	Lt Colonel Kenneth A. Woeber, MC	Alterations in Thyroid Hormone Economy during Acute Pneumococcal Infection.
17 Nov 69 U.S. Senator Thomas J. McIntyre New Hampshire	Colonel Dan Crozier, MC	Medical Defense Against BW.
18 Nov 69 Office of Secretary of Defense, Bureau of the Budget Briefing, Pentagon, Washington, D. C.	Colonel Dan Crozier, MC	Presentation of FY 1971 Budget Requirements for USAMRIID
20 Nov 69 Major Preston, Canadian Forces CBR Liaison Officer	Colonel Dan Crozier, MC	Medical research activities of USAMRIID.

Date and Group or Individual	Individual(s) Participating	Subject
1-2 Dec 69 Annual Command Visit: Colonels Garrison Rapmund, William V. Davis, Robert L. Krivulka; Lt Colonels Donald W. Sample, Richard B. Pedigree, Jr., and Milferd T. Guibor, all of U.S. Army Medical Research and Development Comd.	Colonel Dan Crozier, MC, and Staff	Annual orientation briefing of overall mission and operation of USAMRIID.
8-9 Dec 69 Commanders' Conference, U.S. Army Medical Research and Development Command, held at U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts	Colonel Dan Crozier, MC Lt Colonel Benjamin B. Kirkland, MC	Presentation of USAMRIID program; participants in discussions of command policies and programs.
11 Dec 69 Dr. Bernard T. Tozer Defence Research & Development Staff, British Embassy, Washington, D. C.	Colonel Dan Crozier, MC	Medical Defense Aspects of BW.
11 Dec 69 Briefing for Zarafonetis' Committee, Department of Defense	Lt Colonel Robert W. McKinney, MSC	VEE in Central America.
14 Dec 69 Members of the Frederick County Medical Society	Colonel Dan Crozier, MC Lt Colonel Robert W. McKinney, MSC Major Richard O. Spertzel, VC Captain Duncan S. McGowan, MSC	Tour of Phase I of the new medical facility.
16 Dec 69 Meeting of the American Ordnance Association	Lt Colonel Robert W. McKinney, MSC	VEE in Central America.

Date and Group or Individual	Individual(s) Participating	Subject
19 Dec 69 Rear Admiral John W. Albrittain, Deputy Surgeon General of the Navy; Rear Admiral Ralph E. Faucett, Asst Ch, Bureau of Medicine and Surgery; and other senior Navy Medical officers.	Colonel Dan Crozier, MC	Introduction to Medical Problems.
	Lt Colonel Robert W. McKinney, MSC	Immunoprophylaxis.
	Colonel Joseph F. Metzger, MC	Diagnosis and Identification.
	Dr. William R. Beisel, M.D.	Metabolic Response of the Host.
14 Jan 70 Dietitians' Staff Conference Walter Reed General Hospital	Dr. William R. Beisel, M.D.	Recent Advances in Diabetes Management.
15 Jan 70 Colonels Irvin C. Plough and Kenneth R. Dirks, U. S. Army Medical R&D Command; Colonels William H. Meroney, William S. Gochenour, and Edward L. Buescher, Walter Reed Army Institute of Research.	Colonel Dan Crozier, MC	Coordinate visit and escort individuals for a tour of post laboratory facilities.
20 Jan 70 Dr. A. N. DeSanctis and Mr. James Dodd, President of The National Drug Company, Philadelphia, Pennsylvania	Colonel Dan Crozier, MC, and Staff	Orientation of USAMRIID program and tour of the new medical facility
21 Jan 70 Senior Navy Medical Personnel	Colonel Dan Crozier, MC	Introduction to Medical Problems.
	Lt Colonel Robert W. McKinney, MSC	Immunoprophylaxis.
	Colonel Joseph F. Metzger	Diagnosis and Identification.
	Dr. William R. Beisel, M.D.	Metabolic Response of the Host.

Date and Group or Individual	Individual(s) Participating	Subject
29 Jan 70 Colonel James E. Hansen Commanding Officer, U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts	Colonel Dan Crozier, MC, and Staff	Discussion of command policies and procedures of mutual interest and concern; visit to new medical facility.
2 Feb 70 Gerontology Research Center, National Institute of Child Health and Human Development, N.I.H., Baltimore City Hospitals, Baltimore, Maryland	Lt Colonel Kenneth A. Woeber, MC	Thyroid Activity and Thyroid Hormone Metabolism during Infection in Primates.
7 Feb 70 Armed Forces Institute of Pathology Veterinary Pathology Conference	Captain Ulysses McElyea, Jr.	A Case of Alveolar Cell Carcinoma of the Canine Lung.
9 Feb 70 Military Medicine & Allied Sciences Course, Walter Reed Army Institute of Research	Dr. William R. Beisel, M.D.	Metabolic Responses to Infection.
12 Feb 70 Infectious Disease Seminar University of Maryland School of Med Baltimore, Maryland	Dr. William R. Beisel, M.D.	Lipid Changes in Infection.
19 Feb 70 Armed Forces Epidemiological Board Meeting	Colonel Dan Crozier, MC	Present report of the Commission on Epidemiological Survey for the Commission director.

Date and Group or Individual	Individual(s) Participating	Subject
25 Feb 70 Dr. Jerry Oleson and Dr. Erling Jensen Chas. Pfizer & Co., New York	Colonel Dan Crozier, MC Lt Colonel Robert W. McKinney, MSC Captain Duncan S. McGowan, MSC Mr. Roy C. Culler	Briefing and tour of new medical facility relating to safety features of infectious disease laboratories.
25-27 Feb 70 14th Annual Meeting of Biophysical Society Baltimore, Maryland	Captain William L. Steinhart, MSC	Alteration of Rhythmic Chromatin Template Activity and DNA Metabolism in Livers of Pneumococcus-infected Mice.
	Dr. Anne Buzzell	Metal Chelation by Detergent Michelles: A Model for the Small Pores of Biological Membranes.
9 Mar 70 General Ferdinand J. Chesarek, Commanding General, U. S. Army Materiel Command; Lt General A. W. Betts, Army Chief of Staff for R&D; and M. G. Rouney. Mr. R. L. Johnson, Assistant Secretary of the Army (R&D), Pentagon	Colonel Dan Crozier, MC	USAMRIID FY 1971 budget presentation.
10 Mar 70 Life Sciences Research Program Army Research Office Washington, D. C.	Colonel Dan Crozier, MC Dr. William R. Beisel, M.D. Major Robert A. Massey, MSC	Presentation of the annual technical and budgetary review of USAMRIID program.
31 Mar 70 Veterinary Medical Department, Medical Research Laboratory, Edgewood Arsenal, Maryland	Major David M. Robinson, VC	Canine Viruses Classification.

Date and Group or Individual	Individual(s) Participating	Subject
1 Apr 70 Ad Hoc Committee on Q Fever Walter Reed Army Institute of Research	Colonel Dan Crozier, MC Lt Colonel Robert W. McKinney, MSC	Participants in Ad Hoc Committee Meeting on Q Fever.
	Major David M. Robinson, VC	Investigations of M-44 Strain of Vaccine.
7 Apr 70 Lt Colonel Michael Duffy, MC Medical Liaison Officer to the United Kingdom, Office of The Surgeon General, DA	Colonel Dan Crozier, MC	Medical Defense Aspects of BW
9-11 Apr 70 American Physiological Society Postgraduate Course, American College of Physicians, Philadelphia, Pennsylvania	Lt Colonel Kenneth A. Woeber, MC	The Influence of Binding Proteins on the Distribution and Turnover of the Thyroid Hormones.
12-17 Apr 70 Federation of American Societies for Experimental Biology Atlantic City, New Jersey	Dr. Robert S. Pekarek	Characterization of the Infection-induced Endogenous Mediator of Serum Zinc Depression.
	Dr. Robert W. Wannemacher, Jr.	Significance of Changes in Plasma Free Amino Acids.
	Captain Michael C. Powanda, MSC	Some Aspects of Tryptophan Metabolism in the Rat.
	Major Theodore S. Herman, MC	Effect of Pneumococcal Infection on Hepatic Tyrosine Amino-transferase in Pregnant Rats and their Fetuses.

Date and Group or Individual	Individual(s) Participating	Subject
16 Apr 70 37th Annual Meeting of the American Animal Hospital Association, Denver, Colorado	Major Jerry S. Walker, VC	Clinical Course: Etiology of Tropical Canine Pancytopenia.
26 Apr 70 American Society for Microbiology Boston, Massachusetts	Dr. Robert S. Pekarek	The Endogenous Mediator of Serum Iron Depression during Infection and Endotoxemia.
28 Apr 70 Lt Colonel Dominic Longo, Instructor for C&BW, Medical Field Service School, Fort Sam Houston, Texas; and Colonel L. E. Gutzman, U.S. Army Medical Research & Development Command.	Colonel Dan Crozier, MC	Briefing on current medical doctrine; tour of the new medical facility.
30 Apr 70 Major General Glenn C. Collins, MC Commanding General, Walter Reed Army Medical Center	Colonel Dan Crozier, MC	Visit and tour of the new medical facility.
2-3 May 70 American Federation for Clinical Research, Atlantic City, New Jersey	Major Gordon L. Bilbrey, MC	Renal Function in Acute Pneumococcal Infection.
4-16 May 70 NATO Meeting of the Panel of Experts, Medical Aspects of NBC Warfare, Brussels, Belgium	Colonel Dan Crozier, MC	Participant in meeting of the Editorial Committee on NATO Handbook; participant in TTCP Panel of Experts' Meeting as US Representative on Medical Defense.

Date and Group or Individual	Individual(s) Participating	Subject
14 May 70 Armed Forces Epidemiological Board Meeting	Dr. William R. Beisel, M.D.	Presentation of report of the Commission on Epidemiological Survey to the Board for the Commission Director.
25 May 70 Military Medicine and Allied Science Class Walter Reed Army Institute of Research	Colonel Dan Crozier, MC	Introduction: USAMRIID organization and Mission.
	Dr. Robert S. Pekarek	Trace Metal Change in Infection.
	Dr. Robert W. Wannemacher, Jr.	Amino Acids and Protein Changes in Infection.
	Colonel Joseph F. Metzger, MC	USAMRIID Studies with Staphylococcal Enterotoxins.
	Lt Colonel Robert W. McKinney, MSC	Tour of new medical facility.
	Lt Colonel Peter J. Bartelloni, MC Lt Colonel Harry G. Dangerfield, MC Lt Colonel Robert W. McKinney, MSC Dr. Francis E. Cole, Jr.	Vaccine Research Activities of USAMRIID.
	Dr. William R. Beisel, M.D.	Coordinator of program presented.
26 May 70 45 Thomas Johnson High School Students Frederick, Maryland	Dr. William R. Beisel, M.D.	Coordinator of medical orientation program and tour.
	Major John C. Holder, MC Captain Robert H. Fiser, Jr.	Conductors of clinical medicine tour.

Date and Group or Individual	Individual(s) Participating	Subject
26 May 70 45 Thomas Johnson High School Students (continued)	Captain Donald E. Kahn, VC Captain Jerry D. Weil, VC	Conductors of laboratory animal medical tour.
	Captain Michael C. Powanda, MSC Captain James W. Stiles, MSC Dr. Robert S. Pekarek	Conductors of basic medical sciences tour.
28 May 70 Working Conference on VEE, National Communicable Disease Center, Atlanta, Georgia	Lt Colonel Robert W. McKinney, MSC Major Richard O. Spertzel, VC	Participants in workshop of investigators of the VEE epizootic and epidemic in Central America.
5 Jun 70 University of Maryland College Park, Maryland	Dr. William R. Beisel, M.D.	Trace Metal Studies during Infection.
10-12 Jun 70 52d Meeting of The Endocrine Society, St. Louis, Missouri	Captain John L. Winnacker, MC	Growth Hormone Responses to Acute Pneumococcal Infection in the Conscious Rhesus Monkey.
16-19 Jun 70 1970 Army Science Conference, United States Military Academy, West Point, New York	Colonel Dan Crozier, MC	Participant as Subsession chairman; Co-author on paper presented by Lt Colonel Dangerfield.
	Lt Colonel Harry G. Dangerfield, MC	Biological Effects of SEB.
18 Jun 70 Foreign Animal Diseases Seminar, National Animal Disease Laboratory, Ames, Iowa	Major Richard O. Spertzel, VC	VEE - The Disease and the Recent Outbreaks in Central and S. America
	Major Jerry S. Walker, VC	Rift Valley Fever.

APPENDIX D

PUBLICATIONS OF U. S. ARMY MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES

FISCAL YEAR 1970

1. Airhart, J., G. S. Trevino, and C. P. Craig. 1969. Alterations in immune responses by attenuated Venezuelan equine encephalitis vaccine. II. Pathology and soluble antigen localization in guinea pigs. J. Immun. 102: 1228-1234.

2. Bartelloni, P. J., F. M. Calia, B. H. Minchew, W. R. Beisel, and H. L. Ley, Jr. 1969. Absorption and excretion of two chloramphenicol products in humans after oral administration. Amer. J. Med. Sci. 258:203-208.

3. Bartelloni, P. J., R. W. McKinney, T. P. Duffy, and F. E. Cole, Jr. 1970. An inactivated Eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture. II. Clinical and serologic responses in man. Amer. J. Trop. Med. 19:123-126. Arthropod-borne Virus Exchange 19:134-136, 1969.

4. Beisel, W. R., R. W. Wannemacher, Jr., R. S. Pekarek, and P. J. Bartelloni. 1970. Early changes in individual serum amino acids and trace metals during a benign viral illness of man. Amer. J. Clin. Nutr. 23:660 (abstract).

5. Bilbrey, G. L., R. T. Vollmer, and W. R. Beisel. 1970. Renal function in acute pneumococcal infection. Clin. Res. XVIII:494 (abstract).

6. Buzzell, A. 1969. Ultracentrifugal analysis of metal chelation by polyvinyl toluene latex (PVL) spheres: Application of the phenomenon to biological membrane theory. Program, Third International Biophysics Congress of the International Union for Pure and Applied Physics, Cambridge, Massachusetts, 29 August - 3 September 1969, p. 86.

7. Buzzell, A. 1970. Metal chelation by detergent micelles: A model for the small pores of biological membranes. Biophysical Society Abstracts 10:42a (abstract).

8. Calia, F. M., P. J. Bartelloni, and R. W. McKinney. 1970. Rocky Mountain spotted fever. Laboratory infection in a vaccinated individual. J.A.M.A. 211:2012-2014.

9. Chapple, F. E., III, J. M. Crosbie, B. E. Reisberg, and R. O. Spertzel. 1969. Surgical technique for cross-circulation of rhesus monkeys. Program, 20th Annual Session, American Association for Laboratory Animal Science, Dallas, Texas, 13-17 October 1969, p. 24.

10. Cole, F. E., Jr., and R. W. McKinney. 1969. Use of hamsters for potency assay of Eastern and Western equine encephalitis vaccines. Appl. Microbiol. 17:927-928. Arthropod-borne Virus Exchange 19:132.

11. Craig, C. P., S. L. Reynolds, J. W. Airhart, and E. V. Staab. 1969. Alterations in immune responses by attenuated Venezuelan equine encephalitis vaccine. I. Adjuvant effect of VEE infection in guinea pigs. J. Immun. 102:1220-1227.

12. Crozier, D. 1969. The biological warfare problem. J. Occup. Med. 11:509-512.

13. Crozier, D. 1970. Typhus fevers, p. 72 to 73. In H. F. Conn (ed.) Current therapy, W. B. Saunders, Philadelphia.

14. Dangerfield, H. G. 1970. Tularemia, p. 69 to 70. In H. F. Conn (ed.) Current therapy, W. B. Saunders, Philadelphia.

15. Elsberry, D. D., D. A. Rhoda, and W. R. Beisel. 1969. Hemodynamics of staphylococcal B enterotoxemia and other types of shock in monkeys. J. Appl. Physiol. 27:164-169.

16. Herman, T. S. 1970. Effect of pneumococcal infection on hepatic tyrosine aminotransferase (TAT) in pregnant rats and their fetuses. Fed. Proc. 29:776 (abstract).

17. Howard, R. J., C. P. Craig, G. S. Trevino, S. F. Dougherty, and S. E. Mergenhagen. 1969. Enhanced humoral immunity in mice infected with attenuated Venezuelan equine encephalitis virus. J. Immun. 103:699-707.

18. Irvin, W. S., and C. P. Craig. 1969. Studies of virus alteration of the immune response. Program, 1969 Interim Session, American Rheumatism Association, Tucson, Arizona, 5-6 December 1969. p. 25.

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21. Irvin. W. S., and R. G. Sherry. 1969. The in vitro inhibition of guinea pig macrophage migration by staphylococcal enterotoxin B. Clin. Res. XVII:536 (abstract).

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23. Klainer, A. S., and W. R. Beisel. 1969. Opportunistic Infection: A review. Amer. J. Med. Sci. 258:431-456.

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26. Krisch, R. E. 1969. DNA synthesis by antibody-forming cells during the primary immune response. Nature 222:1295-1296.

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32. Powanda, M. C., and R. W. Wannemacher, Jr. 1970. Some aspects of tryptophan metabolism in the rat. Fed. Proc. 29:569 (abstract).

33. Rhoda, D. A., D. D. Elsberry, and W. R. Beisel. 1970. Fluid compartment alterations in the monkey with staphylococcic B enterotoxemia. Amer. J. Vet. Res. 31:507-514.

34. Rollins, J. B., C. H. Hobbs, R. O. Spertzel, and S. McConnell. 1969. Hematologic studies of the rhesus monkey (Macaca mulatta). Program, 20th Annual Session, American Association for Laboratory Animal Science, Dallas, Texas, 13-17 October 1969, p. 7.

35. Shambaugh, G. E., III. 1969. Tularemia, p. 80 to 82. In H. F. Conn (ed.) Current therapy, W. B. Saunders, Philadelphia.

36. Squibb, R. L., W. R. Beisel, and K. A. Bostian. 1970. Effect of Newcastle disease virus (NDV) on serum diurnal patterns of copper, zinc, cholesterol and carotenoids in chicks. Fed. Proc. 29:695 (abstract).

37. Steinhart, W. L. 1970. Alteration of rhythmic chromatin template activity and DNA metabolism in livers of pneumococcus-infected mice. Biophysical Society Abstracts 10:165a (abstract).

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DISTRIBUTION LIST

US Army Medical Research and Development Command, Department of the Army, Washington, D. C. 20314	5
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DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) U. S. Army Medical Research Institute of Infectious Diseases Fort Detrick, Frederick, Maryland 21701	2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP NA

3. REPORT TITLE

ANNUAL PROGRESS REPORT, FY 1970

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

Annual Report, 1 July 1969 - 30 June 1970

5. AUTHOR(S) *(First name, middle initial, last name)*

Colonel Dan Crozier, MC

6. REPORT DATE 1 July 1970	7a. TOTAL NO. OF PAGES 283	7b. NO. OF REFS 112
8a. CONTRACT OR GRANT NO. b. PROJECT NO. 1B662706A096 c. d.	9a. ORIGINATOR'S REPORT NUMBER(S) RCS-MEDDH-288(R1) 9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*	

10. DISTRIBUTION STATEMENT

Each transmittal of this document outside the Department of Defense must have prior approval of the Commanding Officer, U. S. Army Medical Research and Development Commmand, ATTN: MEDDH-SI, Washington, D. C. 20314

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY U. S. Army Medical Research and Development Command, Office of The Surgeon General, Department of the Army, Washington, D. C.

13. ABSTRACT

A report of progress on the research program of the U. S. Army Medical Research Institute of Infectious Diseases on Medical Defense Aspects of Biological Agents (U) for Fiscal Year 1970 is presented.

KEYWORDS:

Biological warfare
Vulnerability
Therapy
Prophylaxis
Identification
Bacterial diseases
Rickettsial diseases
Host
Parasite
Biochemistry
Pathology
Defense
Metabolism
Virus diseases

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